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JOURNAL OF AGRICULTURAL RESEARCH

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ERRATA AND AUTHOR'S EMENDATIONS FOR VOLUME 35, JOURNAL OF AGRICULTURAL RESEARCH

Page 10, fourth paragraph in summary should be deleted.
Page 32, line 7, "8°" should be "82°."
Page 55, line 2, "heterozygous" should be "homozygous."
Page 55, line 3, "contrary to" should be "in accordance with."
Page 203, line 12, "(12)" should not be italicized.
Page 319, the box heads of the last two columns at bottom of page should be interchanged.
Page 343, Table 10, total dry weight, bottom of column 5, should be ".78" instead of "1.78."
Page 343, Table 10, total dry weight, bottom of column 8, should be ".71" instead of "1.73."
Page 354, Figure 2, add to legend "is a composite diagram from orchard and laboratory observations."
Page 429, footnote 1, line 3, insert "the Department of Zoology" before "Johns Hopkins University."
Page 448, Table 22, footnote symbol "a" should follow "and water" on second line of table heading.
Page 473, legend of Figure 3, "evaginated" should be "invaginated."
Page 550, last line, for "Hall" read "Liberty Hall."
Page 559, Table 2, column 8, line 19, "1.052" should be "0.052."
Page 566, lines 24 and 25, "sheep No. 202" and "sheep No. 201" should read "guinea pig 202" and "guinea pig 201."
Page 632, footnote at bottom of table refers to previous column in each instance—i. e., to the columns for "Ca in urine and feces," "P in urine and feces," and "N in urine and feces."
Page 638, Table No. 1, last number in column headed "Specific gravity" should be "1.0265" instead of ".0265."
Page 639, Table 2, in footnote d, "2 days" should be "3 days."
Page 639, Table 3, column 1, date should be "February 26" instead of "February 21."
Page 658, legend B for Plate 1 should be "Tobacco mosaic on tobacco. X-body (*b*) and nucleus (*n*) in a hair cell. (Nucleus slightly out of focus.)"
Page 830, Table 2, line 3 in third column from right, "4.89" should be "4.80."
Page 844, paragraph 4, line 4, change "B" to "C."
Page 863, line 9, should be "is of low biological value" instead of "if of low biological value."
Page 869, Table 1, row 131, "142" should be "412."
Page 870, Table 1, rows 599 and 710, place entire C. I. Nos. in the column.
Page 914, line 19, "barley in large numbers may not be" should be "barley in large numbers may now be.
Page 1034, Table 8, quality of sauerkraut in vats 3 and 1 should be "fair" instead of "poor."
Page 1034, Table 8, quality of sauerkraut in vats 6 and 4 should be "poor" instead of "fair."
Page 1124, line 27, "suggestions of the" should be "the suggestions of."
Page 1127, lines 4 and 8, change "*batatis*" to "*batatatis*."
Page 1132, Figure 5 is upside down.
Page 1144, fourth line from bottom, delete comma after "considered" and insert comma after "curvilinear."
Page 1145, line 21, after "protein content" delete "for each 1 per cent increase in dark, hard, and vitreou kernels."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 35 WASHINGTON, D. C., JULY 1, 1927 NO. 1

FACTORS AFFECTING CERTAIN PROPERTIES OF A MOSAIC VIRUS[1]

By H. H. McKINNEY

Pathologist in Charge of Cereal Virus Disease Investigations, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Reports on the mosaics of the winter cereal crops (*16*, *18*)[2] indicate that it is difficult to make a systematic study of the virus and of certain important phases of the diseases. That the grass mosaics, under present conditions, are not suitable for use in many of the important fundamental studies of the viruses and of the diseases seems to be very evident. In the case of the winter cereals and corn a large part of this difficulty is accounted for by the fact that these crops do not develop normally under ordinary greenhouse conditions, or in the field, out of their natural growing seasons. Moreover, the virus extracts of all grass mosaics seem to lose their potency rather rapidly after they are expressed by the methods now employed.

A study of the literature on the mosaic diseases of the dicotyledons shows that many of the difficulties which are associated with the grass group are not important and do not constitute serious obstacles in the study of certain mosaics of dicotyledonous plants. As a result, much experimental work has been possible on the properties of the viruses of such mosaics as those of tobacco, cucumber, and tomato.

Practically no work has been done on the properties of the viruses of the grass mosaics. No study has been made of filtration and nothing is known of the tolerance of these viruses to temperature and chemicals, except that inert mineral oil (*6*) and acetone (*9*) areclaimed to favor the extraction of potent virus from mosaic-affected sugar cane.

In connection with the writer's studies on winter-wheat mosaic, it became necessary to determine whether there was justification for carrying out laborious experiments on the wheat plant and the virus in order to reach the point where the properties of the virus might be studied according to the current methods employed in studying the virus or viruses of the tobacco and cucumber mosaics. There was considerable doubt as to whether the results of similar investigations of the properties of the virus of wheat mosaic would be sufficiently trustworthy or significant to warrant the extensive preliminary studies necessary. Moreover, it seemed evident that the proper understanding of the grass mosaics and their viruses depended

[1] Received for publication Nov. 30, 1926; issued July, 1927. Cooperative investigations between the agricultural experiment station of the University of Wisconsin and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 11.

on the solution of certain basic problems which might never be solved to best advantage on the grasses.

Some time ago it was pointed out (*17*) that an investigation should be made to determine the influence of plant extracts on the behavior of viruses. At that time it seemed very doubtful if the differences in the properties of virus extracts obtained from different species of plants, as determined by present methods, could be considered specific for the viruses themselves, and it appeared evident that the value of property studies depended to a great extent on the development of methods which would make it possible to obtain a virus in a more nearly pure state.

The literature indicates that the properties of microorganisms and certain biochemicals are greatly affected by the nature of the dispersing medium and by other factors, such as the number of organisms and the concentration of the biochemical. It seemed entirely possible, therefore, that the properties of such viruses as those of cucumber mosaic (*7*) and tobacco mosaic (*1*) might be explained wholly or in part on the basis of differences between the extracts of the two species of plants and not necessarily on the basis of any specific differences between the viruses.

The writer (*17*) pointed out the desirability of testing this theory by studying the properties of a given virus after passage through different species of plants, and, after an absence of several months from the Wisconsin Agricultural Experiment Station, planned to carry out such experiments on his return to that station. However, after this report (*17*) was submitted it was found that similar studies were just being started at Wisconsin by M. N. Walker. In view of this fact the writer modified his original plans so as not to include the cross-inoculation phases. Accordingly, all of the inoculations reported in this paper were confined to one species of plant, namely, tobacco, and the studies on the effect of plant extracts on the virus were carried out in vitro.

REVIEW OF LITERATURE

Bigelow and Esty (*5*), Esty (*11*), and Esty and Williams (*12*) have shown that the thermal death points of the spores of many thermophilic bacteria isolated from canned foods are influenced by the number of spores, the hydrogen-ion concentration of the medium, and the nature of the medium other than its hydrogen-ion concentration.

Bigelow and Esty (*5*) show in their Tables 4, 5, and 6 that the thermal death points of spores of 19 different thermophilic bacteria are affected by the concentration of the spores when all other factors are uniformly alike. One case, typical of all, may be cited. When the spores of organism No. 26 were heated at 120° C. it was found that the several populations per cubic centimeter were killed in the following periods of time: 40,000 in 12 minutes, 3,800 in 10 minutes, 440 in 9 minutes, and 130 in 7 minutes.

Esty and Williams (*12*) in their Table 1 show that 100,000 spores per cubic centimeter of organism No. 1518, heated at 110° C. in a phosphate mixture of different P_H concentrations, were affected in the following manner: All spores in from 60 to 63 per cent of the test tubes were killed in 20, 22, 40, and 60 minutes when the P_H concentrations were 5.10, 5.55, 6.20, and 7.09, respectively.

Bayliss (*3*), Euler (*13*), and others point out that the properties of enzymes also are affected by their concentration. Euler states that the action of such paralyzers as chloroform depends to a great extent on the concentration of the enzyme. It is considered that the injurious effects on the enzyme are greater as its concentration decreases. Bayliss and also Euler point out that the properties of enzymes are influenced by colloidal impurities, salts, and proteins present in the extracts. O'Sullivan and Tompson (*21*) found that sucrose extract is completely inactivated near 55° C. in the absence of sucrose, but when sucrose is present the enzyme was only partially inactivated near 75°. Bayliss (*2*) found that the presence of charcoal in an extract of trypsin increased the thermal tolerance of the enzyme.

Wells (*23*) indicates that the properties of toxins and antitoxins are affected by many substances and that a given property of a toxin or antitoxin is not necessarily fixed under all conditions.

From these citations it is evident that some of the properties of microorganisms and of biochemicals do not remain constant under all conditions. Thus the assumption that the properties of a virus may be affected by its concentration and by constituents in the extract has some support. To test this assumption, the following experiments were conducted with the virus extract from tobacco affected with mosaic: (1) Determination was made of the effect of the virus concentration on (*a*) the thermal inactivation point and (*b*) the potency of the virus while stored at room temperature; (2) the effect of such diluting media as water and cucumber and tobacco fluids was determined on thermal inactivation and on potency of the virus while standing at room temperature.

METHODS

The virus was obtained originally from a single young mosaic affected tobacco plant which was kindly supplied the writer by James Johnson and M. Mulvania. The mosaic pattern on this plant [3] was comparable to that illustrated by Iwanowski (*14*) and, although the mottled leaves showed no deformities, many of the plants subsequently inoculated with this virus developed leaf deformities similar to those illustrated by Iwanowski.

This plant was handled with great care to avoid any contamination with other viruses. The hands were washed in 95 per cent alcohol and dried immediately before the plant and the fluid were handled. The plant was ground to a pulp in a sterilized food chooper. The pulp was collected in sterile gauze placed in a sterile dish and the fluid was then pressed out by hand. Part of the fluid was put in a sterile test tube. This was put away and safeguarded against any

[3] It has been pointed out by the writer in a brief article (*19*) that the virus of this plant regularly causes small, yellow spots on older plants affected by the typical "green mosaic." The virus obtained from these isolated yellow spots produces "yellow mosaic" in young plants, and by successive subtransfers of virus from yellow spots the proportion of yellow mosaic can be increased to a point where it dominates over the green mosaic throughout the complete life cycle of the plant.

Five additional lots of tobacco-mosaic virus were obtained from widely separated regions in the United States, and another lot was obtained through R. J. Riker from England. When plants were inoculated with these viruses it was found that the same type of yellow spots developed in older plants and that all were like the spots described above. In one case fresh leaves which were especially well preserved while in transit, showed a yellow spot on being received from the sender. This spotting usually develops when the plant has produced from 12 to 15 leaves. All of the evidence now in hand indicates that the association of these viruses may not constitute a contamination in the usual sense. A more complete report will be made of this work later.

possible contamination from another virus. The few plants which were inoculated in the beginning supplied virus for subsequent experiments.

In all experiments the virus and plant extracts were obtained by grinding mosaic-diseased or mosaic-free plants in a mortar with quartz sand. The fluid was obtained by pressing the pulp in a screw press or by hand. Although the fluids were not filtered, all coarse tissue was removed by passing the fluid through several thicknesses of finely woven cotton gauze. Dilutions were made immediately with sterile distilled water or with fresh fluid from mosaic-free tobacco and cucumber plants. Dilutions were not carried beyond a point (one thousand times) which would produce 100 per cent infection with fresh unheated infectious juice. All glassware, grinders, gauze, or other materials which came in contact with the virus were sterilized before being used.

In all of the experiments on thermal inactivation, the extracts were placed in pieces of glass tubing which were 7 mm. in diameter (inside) and 45 mm. long, 0.8 c. c. of extract being placed in each tube. The tubes were closed at both ends by means of small, tightly fitting corks. These were held tight by means of a stout cord. The temperatures were maintained by an insulated water bath 6 inches deep and 8 inches in diameter, and the temperature was determined by means of a standardized thermometer. The temperatures were regulated within less than 0.2° C. by means of a hand-regulated gas flame. The tubes were completely immersed and held in the center of the bath on a wire tray, which also served as an agitator. The temperature of the water was taken at the level of the tray. Fifteen seconds were allowed for the extracts to reach the temperature of the water. As soon as the tubes had been subjected to the proper temperature for the prescribed time, they were all removed at once and plunged into cold water immediately.

This method was adopted after a study had been made of the methods commonly employed. It is natural to expect greater regularity in heat penetration of substances in small containers which are completely immersed than in test tubes which are not completely immersed. This point seems to be borne out by Bigelow's (*4*) studies on heat in the canning industry. Most bacteriologists use ampules of small diameter, drawn and sealed at the ends in a gas flame. The writer employed this method, but it was discarded and the corked-tube method was substituted. The continued use of the method has proved it to be very convenient and simple for studies of viruses, as temperatures do not go above 100° C.

In the studies on the reduction of the potency of the virus while standing at room temperature, each lot of virus extract was diluted with the prescribed quantity of sterile, distilled water, or tobacco or cucumber extracts. The resulting fluids were put in sterile test tubes, which were then corked and stored in the laboratory. This method might have been refined somewhat by using filtered fluids. However, as unfiltered juices have commonly been employed by those studying the properties of viruses, and as quantitative studies (*20*) show that filtered juice usually has a very low virus concentration, it was decided to use unfiltered fluids in these experiments.

In all of the experiments the plants were cultured and inoculated in accordance with the methods described in the accompanying

paper (*20*). The writer kept close watch for any variation in symptom manifestations which might be caused by heating or diluting the virus. The mosaic symptoms of different plants which have been subjected as nearly as possible to the same conditions do vary somewhat, and there is also some variation due to different environmental conditions, but, after taking these variations into account, the writer has not yet seen additional symptom variations which seemed to result from the treatments of the virus. In some cases mild forms of mosaic appear in the early stages of the disease. However, in all such cases resulting from heated virus, the writer has held the plants for a longer period, and in every instance such plants have soon developed typical mottling of a rather severe type. All of the evidence obtained thus far clearly indicates that critical studies can not be made in mosaic symptomatology entirely on young stock. Mosaic-affected plants must be held for relatively long periods, sometimes until flower-bud development, if a complete story of symptom types is to be obtained.

Some investigators object to holding plants for long periods because of the possibility of their becoming accidentally infected with other viruses. This objection is begging the question. Precautions must be employed which make it safe to follow this method.

RESULTS

The data obtained in the studies on thermal inactivation are presented in Tables 1 to 4. From these data it will be seen that at the higher temperatures the potency of the virus becomes less as the concentration of the virus is reduced. It will be seen also that this general relationship holds consistently for all of the diluting media used. However, it is evident that the degree of reduction in the infectious activity was influenced by the diluting medium. In all experiments the uninoculated control plants remained free from mosaic symptoms.

TABLE 1.—*Percentage of tobacco plants that developed mosaic in three experiments in each of which five plants were inoculated with virus extract undiluted or diluted 10, 100, or 1,000 times with sterile distilled water, and held at 80° or 85° C. for different periods*

Time virus was exposed to 80° C.	Percentage of mosaic at dilutions of—			Time virus was exposed to 85° C.	Percentage of mosaic at dilutions of—		
	0	100	1,000		10	100	1,000
5 minutes	100	100	60	5 minutes	100	80	60
20 minutes	100	100	0	10 minutes	100	60	20
Unheated control	100	100	100	15 minutes	100	20	0
5 minutes	100	100	100	20 minutes	100	0	0
20 minutes	100	80	40	Unheated control	100	100	100
Unheated control	100	100	100				

In some cases, heated virus extract diluted one hundred times caused 100 per cent infection. However, except in one case, infection was reduced by heating virus diluted one thousand times. The one exception is shown in Table 1, where the virus was heated for but five minutes at 80° C. It is not surprising that 100 per cent infection

was produced by some of the heated extracts diluted one hundred times, as the unheated virus diluted one thousand times always produced 100 per cent infection, except when cucumber fluid was used. Furthermore, heat treatments at 80° do not appear to be sufficiently severe to produce marked injury with regularity. Doubtless quantitative tests would have shown that the potencies of the viruses in the dilutions of one hundred times and in the exception cited above were actually reduced by the heat treatments, even though this reduction was not sufficient to show in these tests.

TABLE 2.—*Percentage of tobacco plants that developed mosaic when five plants were inoculated with virus extract undiluted or diluted 100 times with sterile distilled water, and held for 10 minutes at different temperatures*

Temperature for 10 minutes (°C.)	Percentage of mosaic at dilutions of—		Temperature for 10 minutes (°C.)	Percentage of mosaic at dilutions of—	
	0	100		0	100
82	100	40	90	0	0
84	100	0	92	0	0
86	100	0	Unheated controls	100	100
88	80	0			

TABLE 3.—*Percentage of tobacco plants that developed mosaic when five plants were inoculated with virus extract undiluted or diluted 10 or 100 times with sterile distilled water or with tobacco extract* [a]

Temperature for 10 minutes (°C.)	Dilution magnitudes and percentages of mosaic					
	Water dilutions			Tobacco-fluid dilutions		
	0	10	100	0	10	100
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
80	100	100	40	100	100	100
84	100	100	20	100	80	80
88	100	0	0	100	40	0
Unheated controls	100	100	100	100	100	100

[a] Although the plant from which this fluid was obtained showed no signs of mosaic, the fluid contained a very small trace of virus, as one out of five control plants inoculated with this undiluted fluid developed mosaic.

TABLE 4.—*Percentage of tobacco plants that developed mosaic when five plants were inoculated with virus extract undiluted or diluted 10, 100, or 1,000 times with tobacco or cucumber extract*

Temperature (°C.) for 10 minutes	Dilution magnitudes and percentages of mosaic						
	Tobacco-fluid dilutions				Cucumber-fluid dilutions		
	0	10	100	1,000	10	100	1,000
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
85	100	100	20	0	0	0	0
Unheated controls	100	100	100	100	60	40	0

In order to obtain some idea of the influence of the virus concentrations on the temperature at which inactivation occurs, an experiment was carried out with fresh, undiluted extract and with the same extract diluted one hundred times in water. This dilution is very low and has never failed to produce 100 per cent infection. These extracts were then subjected to a series of 10-minute treatments at temperatures ranging from 82° to 92° C. The results shown in Table 2 indicate that the thermal inactivation point dropped at least 5° when the virus was diluted one hundred times. It is fully recognized that the extract in each dilution varied with the concentration of the virus, and this doubtless had some influence on the results. However, this point can not be accurately determined until it is possible to study a purified virus in a uniform extract.

A comparison of the data in Tables 1 and 2 shows that the virus extract used in the 85° C. heat series, which was diluted one hundred times, caused 60 per cent of mosaic when heated 10 minutes (Table 1), whereas the virus of the same dilution when heated at 84° and 86° for 10 minutes caused none (Table 2). This difference in the behavior of the virus can not be explained at the present time. It is possible that the two viruses differed considerably in their initial concentrations. It is also likely that the fluids in the different plants varied. Observations show that the physical and chemical nature of the fluids vary greatly in plants grown under different conditions. The coagulation temperature of the proteins in the fluid seems to vary considerably, and the precipitation of coagulated materials also varies greatly. Any or all of these variables doubtless affect the thermal inactivation and other properties of the virus. It therefore becomes apparent that attention must be directed toward the more accurate control of growth conditions in order that reasonably uniform plant fluid may be obtained.

From the data presented in Table 4 it is noted that dilution with fluid from healthy cucumber plants had a very depressing effect on the thermal inactivation of the virus of tobacco mosaic. Cucumber fluid also reduced the infectiousness of the unheated virus. From these results it seems entirely possible that the relatively low thermal inactivation point of the virus of cucumber mosaic may be explained in part on the basis of the nature of the cucumber-plant fluid.

The experiments dealing with the effect of time and of different diluting media on the potency of the virus were not repeated as many times as were the experiments relating to temperature and dilution. However, in general, the results seem to be sufficiently consistent to warrant presentation in Table 5.

From these data it is apparent that after standing, the virus became less infectious as its concentration was reduced. In general, it appears that the healthy tobacco and cucumber fluids, especially the latter, had a little more depressing effect on the potency of the virus than did sterile distilled water. These results and those in Table 4 make it seem that the short period over which the expressed virus of cucumber mosaic retains its infectious property also may be due in part to the nature of the cucumber-plant fluids.

TABLE 5.—*Percentage of tobacco plants that developed mosaic in four experiments in each of which five plants were inoculated with unheated virus extract undiluted or diluted 10, 100, or 1,000 times, with distilled water, tobacco, or cucumber extracts* [a]

Time (days) virus stood in laboratory	Dilution magnitudes and percentages of mosaic									Undiluted virus
	Water dilutions			Tobacco-fluid dilutions			Cucumber-fluid dilutions			
	10	100	1,000	10	100	1,000	10	100	1,000	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
2	100	80	60	100	80	20	100	20	0	100
6	100	100	80	100	100	80	100	80	0	100
12	100	80	80	80	60	40	80	60	0	100
15	100	40	40	100	40	20	100	40	20	100
Means	100	75	65	95	70	40	95	50	5	100

[a] The dilution samples stood in the laboratory in corked test tubes during the period of the experiments.

It is noted from Table 5 that the virus diluted more than ten times tended to cause a higher percentage of infection after it had stood for six days than it did at the end of the second day. When the virus was diluted one thousand times in cucumber fluid the infection was greatest after 15 days' standing. In general, after the sixth day the infection curves for virus diluted above ten times tended to drop.

DISCUSSION

In somewhat similar experiments, Elmer (*10*) observed that infection from diluted virus of tobacco mosaic was first depressed and later increased. However, he worked only with filtered fluid and with dilutions of ten times. Also, his studies were conducted over a shorter time period than were the writer's. In the present studies, the plant fluids had little effect on the virus when diluted ten times. Elmer's results with virus diluted ten times are probably explained in part on the basis of a very low initial concentration of the virus. It seems likely that the virus used in Elmer's experiments was from fifty to one hundred times less concentrated than virus employed in the present studies.

Indications of an accelerating or reactivating influence on the potency of the virus has been evident in several of the writer's experiments. However, it appears now that much study is necessary before it will be possible to distinguish with certainty between what may be experimental variation and actual reactivation of previously inactivated virus through colloidal and adsorption changes, or through an actual increase in the number of virus particles not previously inactivated.

Granting for the moment that this apparent reactivation of the virus may not be due entirely to experimental variation, it is of interest to consider the behavior of certain biochemical substances. From a review of the literature on enzymes and toxins, it appears that these substances vary greatly in their ability to dissociate or disperse as the adsorption relationships are altered in their extracts. There is considerable evidence which indicates that inactivation

does not necessarily signify destruction. Under certain conditions, these substances may be reactivated after having been inactivated, or partially inactivated. The expressed viruses of plants are associated with many other materials in the fluid, and when the fluid is allowed to stand it undergoes many changes. Under such conditions the reaction and adsorption relationships doubtless are altered many times, and it seems quite likely that these fluctuations may affect the quantities of free virus from time to time. While it may be that we are dealing with an organism which, under certain circumstances, increases in vitro, it seems apparent that it is not necessary to consider that the virus is an organism in order to explain the results cited.

Duggar and Armstrong (*8*) have reported that the fluids from the pokeweed inhibit the infectiousness of the virus of tobacco mosaic in vitro. They tested the effect of the juices from many plants on the virus and found that some of them did not seriously reduce its infective power. The writer carried out similar experiments with pokeweed juice and obtained the same results. In addition, several tests were made in which tobacco plants were inoculated with the fresh virus extract of tobacco mosaic, and soon afterward the same plants were reinoculated with the fresh extract from healthy pokeweed plants. In another set of experiments the plants were first inoculated with the pokeweed juice and then inoculated with the virus extract. In all of these cases, when the pokeweed and virus extracts were not mixed in vitro before making the inoculations, mosaic occurred in 100 per cent of the inoculated plants.

It is not surprising that certain plant extracts are toxic to a virus in vitro. Our most deadly poisons in many cases are of plant origin and it would be indeed surprising if some of these are not found to be very toxic to many substances in other species of plants. Only by further studies can it be determined if the toxic principle is in the pokeweed juice or if there is a new or secondary substance produced by a combination of substances in the pokeweed and tobacco extracts which is toxic to the virus.

Since the preparation of the present paper, the results of Walker's (*22*) studies have been published, and it is evident that his results also indicate rather clearly that different plant extracts affect differently the properties of a virus.

It may be suggested that the different experiments presented here should have been repeated many more times than was the case. The writer fully appreciates the value of repetition in experiments provided uncontrolled variables are not in excess of those which are controlled. It is believed that there are many uncontrolled variables in connection with the study of plant extracts and it is puzzling to know how best to control all of them.

The data presented seem to be very consistent in that they show no excessive irregularities. This in itself strengthens the results, and it is believed that more is to be gained from a study of the uncontrolled variables in plant extracts than would be gained from continued repetitions of the experiments made.

It is entirely possible that fluids from mosaic-free tobacco and cucumber plants may affect the properties of a virus somewhat differently than do the fluids from these plants when severely affected with mosaic. It is also fully recognized that in the experiments

reported here the virus concentration and type of diluting medium were unavoidably varied simultaneously. However, after taking these points into consideration, it appears that the data presented indicate rather clearly that the thermal inactivation of a virus and the time period over which it retains its potency at room temperature, are affected by the concentration of the virus, and also by the plant constituents in which the virus is contained.

It further appears from these data that the results of comparative studies on the properties of viruses causing mosaic on different species of plants can not be interpreted accurately until the viruses can be studied in nearly equal concentrations and in some standard fluid. It is possible to approach a uniform fluid by transmitting the viruses of different mosaics to some given host from which the virus-containing fluids can be expressed for detailed property studies. However, it is believed that the introduction of plant constituents associated with a virus into a given host may complicate such a method. This belief seems to have some support in the results obtained by Johnson (*15*) with the fluids of supposedly healthy potatoes. When the potato-plant fluids were introduced into healthy tobacco plants, an infectious disease resulted.

In the end, it would seem that we must look forward to the purification or isolation of the virus. Although several obstacles seem to be in the way of this accomplishment, it is believed that progress can be made in this direction. One of the chief obstacles has been that no adequate quantitative method was available for the estimation of the concentrations of viruses. However, it is believed that preliminary results, which are published in the accompanying paper (*20*), show that dilution and inoculation tests can be standardized to a point which will enable the detection of relatively small differences in concentration.

SUMMARY

The temperatures at which the virus of tobacco mosaic become inactivated depends on the concentration of the virus and on the nature of the plant extract.

Virus extract diluted one hundred times with water was inactivated in 10 minutes at a temperature between 82° and 84° C., whereas the same virus undiluted was inactivated at a temperature between 88° and 90°.

Fluid extract from healthy cucumber plants seems to have a more depressing effect on the potency of the virus than does an equal amount of water or tobacco fluid.

Virus diluted with water or tobacco and cucumber juices loses its potency more rapidly on standing than does undiluted virus extract. This is seemingly more marked when the dilutions are made with healthy cucumber extract.

The first reduction in the potency of diluted virus extract does not appear to be permanent in all cases. The nature of this reactivation may be the result of an adsorption phenomenon or of an actual increase in the number of virus particles which were not previously inactivated.

The properties of viruses from different species of plants can not be studied adequately in a comparative way until the viruses can be purified, brought to a fairly uniform concentration, and studied in reasonably uniform dispersing media.

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QUANTITATIVE AND PURIFICATION METHODS IN VIRUS STUDIES[1]

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INTRODUCTION

Studies on the viruses of the mosaic diseases indicate that much of the extraneous material must be removed from the virus extracts before it will be possible to carry out many important experiments in a satisfactory manner. In attempting purification, several methods are available, but, owing to the relatively small amount of study which has been directed toward this problem, little is known concerning the practicability of the methods.

On the assumption that the virus of mosaic is an independent organism, several workers have attempted to cultivate it, and although Olitsky (*20*)[3] claims success with the virus of tobacco and tomato, Mulvania (*19*) and Purdy (*22*) failed to verify his results. It is possible that under very special conditions which were not apparent to Olitsky, the virus will increase or become more potent in vitro. However, as pointed out in the accompanying paper (*17*), it seems to be unnecessary to assume that the virus is an independent organism in order to explain such results as were claimed by Olitsky. Although it is possible that the virus may be isolated by pure-culture methods, it appears that some attention should be directed toward physical and chemical methods of purification.

Several theories have been advanced regarding the nature of the mosaic virus, but none of our present methods seems to be adequate for testing these theories in a satisfactory manner. Several of the most basic methods which are required in the work will be applicable regardless of the nature of the virus, and it seems apparent that their best development is not dependent on the acceptance of any one of the present theories concerning the virus.

In attempting the purification of the virus by any method it is soon found that little progress can be made until a quantitative method is available. Such a method is necessary for determining the relative concentrations of virus in physical and chemical fractions as well as in culture solutions.

QUANTITATIVE STUDIES

Although it is entirely possible that one might develop a chemical or a microscopical method which would be infallible for testing the concentration of the virus, it seems somewhat more hopeful at

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[2] The writer wishes to express his appreciation to R. W. Webb for assisting in certain of the inoculations in experiments in which large numbers of plants had to be inoculated on any one day, and for helping in other phases of the work.

[3] Reference is made by number (italic) to "Literature cited," p. 37.

present to attempt the development of a method based on a biological assay, that is, the occurrence of disease symptoms.

The use of biological tests for quantitative determinations is not new. In the standardization of toxins and antitoxins, such methods have long been in use. Ehrlich (*6*), in his classic studies in immunology, found that he could standardize the concentration of diphtheria toxin and antitoxin by employing test animals, and his "minimal lethal dose," the minimum amount of toxin which will be sure to kill a normal guinea pig weighing 250 gm. in a period of four days, is still the basis for standardizing this toxin. Modifications of this method are also used for standardizing several bacterial toxins and vaccines.

It has been pointed out by Drummond and Watson (*4*) that the detection and estimation of vitamins in foods can best be accomplished through the use of biological tests in spite of the errors involved. In testing for the concentration of vitamins (*7, 8, 25*) the test animals are fed gradually increasing amounts of the test food, and the relative concentrations of the vitamin are determined on the basis of the behavior of the animals. Similar methods are also used in the standardization of certain drugs (*2, 9, 21*).

In view of the fact that several investigators have found that the mosaic viruses can be diluted to a considerable degree without completely losing their potency, it appeared to the writer that a system of dilution experiments might serve as the best basis for preliminary quantitative studies. The three following factors offered possible means for measuring relative concentrations of a virus: (1) The length of time elapsing between inoculation and the appearance of disease symptoms; (2) the degree or the intensity of the symptoms; and (3) the percentage of inoculated plants which develop mosaic within a given period.

In the studies on the scab of potato, the disease caused by *Actinomyces scabies* (*11*), and in those on the wheat foot-rots caused by *Helminthosporium sativum* (*15*), and *Ophiobolus graminis* (*18*), the writer observed that the number of infected individuals and the severity of the injury varied as the amount of inoculum used in the different experiments was varied. The literature on the mosaic diseases indicates that the number of plants which develop mosaic after inoculation also varies with the concentration of the virus (*1, 3*). However, it is now evident that the methods employed previously do not make it possible to detect differences in the concentration of virus in most extracts until dilutions ranging from 1,000 to 50,000 times are reached. Obviously, this is a disadvantage, and in view of the importance of detecting differences between more concentrated virus extracts and of the desirability of having some reasonably uniform quantitative method, it was necessary to carry out new experiments designed to furnish the required information.

Although Walker (*24*) considers that the dilution property is very irregular and unsatisfactory for purposes of comparing viruses from different species of plants, it became evident to the writer, after completing several experiments, that the dilution method can be used for quantitative work. However, success with the method depends on certain important factors and on methods which can be standardized to a reasonable degree.

(1) The virus must lend itself to study in the expressed fluids without losing its potency in a short time.

(2) The plant employed must be easily cultured under greenhouse conditions during as much of the year as possible.

(3) It must be highly and uniformly susceptible to the disease, and must develop definite symptoms.

(4) The plants used in a single experiment must be grown under the same conditions.

(5) Growing conditions for all experiments must be standardized as far as possible.

(6) Accidental infection through insect carriers, soil, contact with infected or infested materials, must be guarded against.

(7) The inoculation technic employed must lend itself to the greatest possible uniformity and certainty.

In considering the plant and the virus most suitable for the quantitative studies, it became evident that none of the cereals or other grass crops, or their mosaics, could be employed at the present time. While there are several dicotyledonous hosts which might have been used, none seemed so well adapted for the studies as the tobacco plant and the virus of its mosaic disease.

PLANT-CULTURE METHODS

Soil

Early in these studies it was observed that cases of accidental mosaic sometimes occurred in experimental stock when there was no evidence of insect or accidental contact transmission through the above-ground parts. Since the writer (*14*, *16*) had found that wheat mosaic may be transmitted through the soil, limited studies were made to determine whether tobacco mosaic might be transmitted in the same way.

The virus-free soil used in these experiments was obtained from an old wood lot near Madison, Wis. Previous tests had shown that the soil produced healthy plants. The seedlings were grown in this soil in flats. When sufficiently large they were transplanted to the experimental soils in 4-inch earthen pots which had been sterilized with steam.

TABLE 1.—*Number of plants showing tobacco mosaic in soil receiving different treatments*

Soil treatments	Number of plants tested	Number of mosaic plants
Experiment 1:		
Virus-free, control	20	0
Mosaic tissue and juice	19	14
Roots from mosaic plants	20	6
Experiment 2:		
Virus-free soil, control	24	0
Virus-free sand, control	24	0
Sand from beneath pots of mosaic plants	23	0
Virus soil, control	23	4
Virus soil+formaldehyde	24	0
Virus soil+uspulun (5 gm.)	24	6
Virus soil+uspulun (10 gm.)	24	4

In experiment 1 a series of seedlings was planted in virus-free soil; a series in soil in which mosaic-affected tobacco had recently grown, and which still contained the fresh roots of these plants; and a series in virus-free soil to which had been added, a few days previous to transplanting, juice and pulp from mosaic-affected tobacco plants. The results of experiment 1 (Table 1) indicate that infection does occur from the soil in a fairly large number of plants.

Experiment 2 was conducted to obtain some idea as to methods of controlling infection from the soil, and to determine whether potted plants would become diseased if placed on bench or floor sand on which potted mosaic plants had previously grown. The virus-free soil was of the same lot as that employed in experiment 1. The virus-infested soil was obtained from pots in which mosaic-affected plants had been growing. The fresh roots were present in the soil. The supposedly infested sand was obtained from a greenhouse bench on which mosaic-affected plants had been growing in pots immediately previous to the removal of the sand. The results of experiment 2 (Table 1) likewise show that infection may originate from the soil.

Johnson (*10*) has shown that tobacco does not grow well in many soils which have been sterilized with steam just before the introduction of the seedlings, and the writer's experience bears out this observation. Accordingly, chemical disinfectants were used instead of steam. As the writer had found previously that wheat moasic can be controlled effectively when virus-infested soil is treated with a solution of 1 part fresh 40 per cent formaldehyde solution in 49 parts water, this treatment was employed. In addition, uspulun was applied in two concentrations, 5 gm. to a liter of water and 10 gm. to a liter of water. In all cases the infested soil was made into a stiff mud by the addition of the disinfectants, and by water in the case of the soil control. These soils were held in covered containers for 30 hours, and were then spread in layers and dried until no fumes of formaldehyde were noticeable from those treated with that chemical. They were then potted and the seedlings were transplanted into them.

Virus-transmitting insects which feed on the above-ground portions of the plants were guarded against. Very few insects were present outdoors during the period of these experiments and none were noted in the greenhouse in which the experiments were conducted. The uninoculated control plants were kept close to those planted in infested soil, and as they remained free from mosaic it is only reasonable to consider that the mosaic which occurred in the remaining plants had its origin from virus present in the soil.

From these preliminary tests it appears that soil treatments with freshly manufactured formaldehyde prevent tobacco-mosaic infection from the soil as effectively as they do wheat-mosaic infections from the soil, and that this disinfectant is more effective than uspulun in the concentrations employed.

From the one experiment it appears that bench sand may not be a serious source of accidental infection. This result is in harmony with field observations (*16*) in which the writer has found that wheat mosaic does not persist for as long a period in the sandy soil of the Illinois River region as it does in the heavy silt soil in the Mississippi River region near Granite City, Ill.

As a result of the experiments cited, every precaution was taken in the greenhouse studies to prevent accidental infection from the soil. If virus-free soil could not be obtained readily, other soil was disinfected with fresh formaldehyde. In all cases pots were sterilized with steam. Although it is possible that virus which gets into the sand under pots may in some cases cause accidental infection, it seems likely that most of this is washed deep enough to get below the few roots which may come through the pots in experiments of comparatively short duration.

If pots are placed in beds in the center of a house, it is well to set them on a layer of fine gravel several inches deep. By thoroughly wetting the gravel daily, and by disinfecting it several times a year, the possibility of accidental infection of young stock seems to be reduced to a minimum.

Since all possible infection from the soil and from other agencies has been guarded against, the number of accidental cases of mosaic has been noticeably reduced. In fact, accidental infection is so rare that it causes practically no concern.

Plant Stock

In the early experiments several varieties of tobacco were used. However, as the work developed all of the studies were confined to one strain of Connecticut Havana. The material from which the seed was obtained was kindly supplied by James Johnson. The seed used subsequently was derived from a single plant of this lot, growing in the field. In order to guard against possible cross-pollination, the plant was bagged before the flower buds opened. In all subsequent seed propagation the same precautions were taken. The seed from different plants was never mixed. It is believed that by following these methods all irregularities in infection due to genetic causes in the host were reduced to a minimum. In the winter the temperature in the growing house was kept as near 75° to 80° F. as possible. In the summer, temperatures fluctuated with the weather.

In all cases single plants were grown in 4-inch earthen pots. From 5 to 30 plants were inoculated with each sample of diluted virus extract, and all sets of plants inoculated with a given dilution were uniform in size and age. The plants were inoculated just before or during the period of their greatest vegetative development. In all of the earlier experiments healthy control plants were carried along with the inoculated plants, and in no case did mosaic occur in any of these during the first two weeks of an experiment. All experiments were discontinued at the end of this period.

Inoculation Technic

At the outset of the work it was found necessary to develop a method of inoculation by which it would be possible to obtain 100 per cent infection when the plants were inoculated with fresh, undiluted virus extract. After some experimenting, it was found that the percentage of mosaic plants resulting from inoculation with highly diluted extract increases as the amount of inoculum introduced into the plant is increased. To compare the results on a quantitative basis, it therefore becomes necessary to introduce as nearly as possible the same amount of diluted and undiluted fluid

into each plant in a given experiment. Owing to the nature of the vascular elements in tobacco, it is difficult, if not impossible, to inject a definite and constant quantity of fluid into the vascular system and have it all remain there. When a small hypodermic needle is inserted into the tissues practically all of the fluid injected comes out through the wound made by the needle.

After employing several methods of inoculation, the following was found both expedient and reasonably consistent in its results. A uniform amount of absorbent cotton lint was wrapped on the point of a stout, polished, steel needle. To this was applied several drops (one-eighth c. c.) of infectious fluid by means of a finely drawn glass pipette, or else the lint-tipped needle was dipped directly into the fluid. Either method gave satisfactory results. The saturated cotton was applied to the axil of a leaf near the base of the plant. The cotton was pushed down into the vascular region of the stem and petiole by means of the needle. The stem, petiole, and ligule were pricked and scratched at several points through the fluid which remained in the axil. Each inoculation needle was disinfected in 95 per cent alcohol, rinsed in water, and wiped before it was used to inoculate another plant. By using several needles the disinfection process caused no great inconvenience. Early in the work the operator's hands were washed in 95 per cent alcohol and wiped dry before an uninoculated plant was touched, but as the work progressed this operation seemed unnecessary, and it was employed only when proceeding from one lot of virus extract to another, or when it was necessary to handle some object which might be contaminated with a virus. Figure 1 illustrates the inoculation method.

Since using this method the writer has never failed to obtain 100 per cent infection in the many hundreds of plants inoculated with undiluted, fresh virus extract. Only in rare cases has he failed to obtain 100 per cent mosaic in plants inoculated with fresh extracts diluted 1,000 times, and in these cases there was evidence that the concentration of the virus was unusually low in the plants selected for the inoculum.

EXPERIMENTS

In the first experiments the writer inoculated five plants each of several series with lots of undiluted virus or with virus diluted 1,000, 10,000, 50,000, and 100,000 times in sterile distilled water. It was soon observed that the type of mosaic symptoms occurring on the plants inoculated with the virus in different concentrations varied so little, and these variations were so inconsistent or so difficult to measure accurately, that this factor could not be used to advantage in quantitative studies. In some plants a very mild type of mottling occurred. Several such plants were kept after closing an experiment, and in all cases they developed typical mottling as they continued to grow.

Although the time elapsing from inoculation to the appearance of mosaic was a little shorter for plants inoculated with the higher concentrations of virus, there was not sufficient regularity with respect to this manifestation to render it of any great value in quantitative studies. The most striking and consistent variable was the number of plants which developed symptoms within 4 to 15 days after inocula-

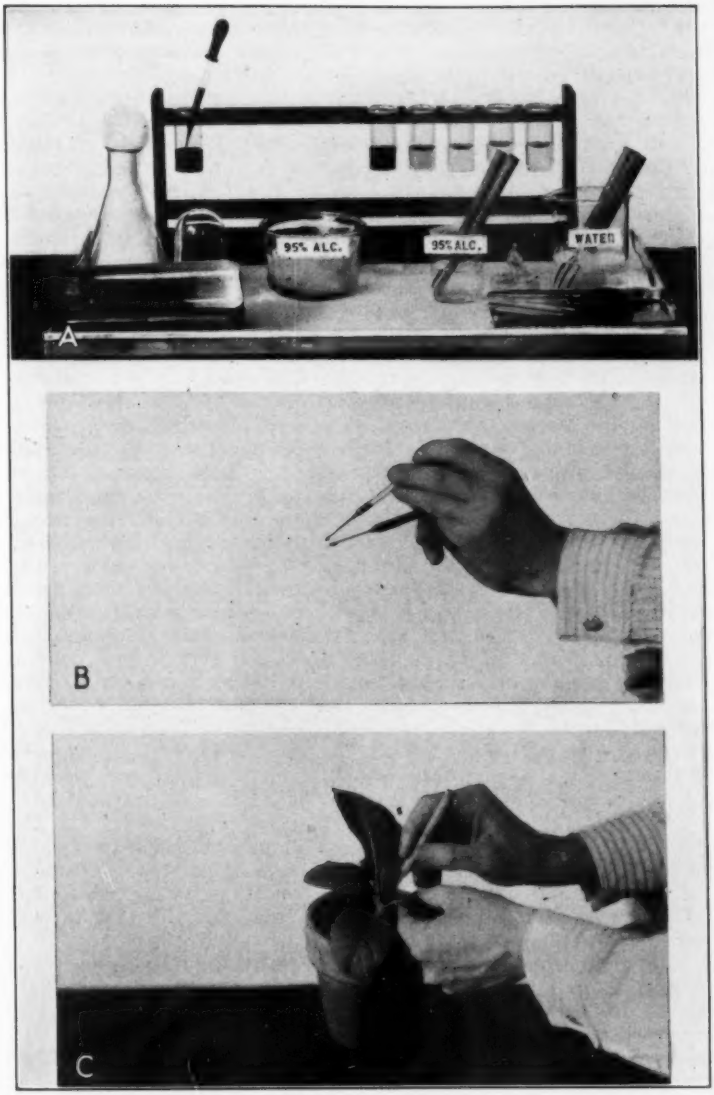

FIG. 1.—Materials and method used in inoculating the test plants: A, arrangement of the dilution samples and other items used for inoculating; B, method used for applying inoculum to the cotton on the tip of the needle (equally good results have been obtained by dipping the cotton in the extract contained in the vials, provided the fragments of cotton are of approximately the same quantity and compactness); C, method used in pushing the cotton and inoculum into the tissues at the juncture of the stem and the leaf petiole

tion. As this relationship offered promising possibilities for measurement, other similar experiments were carried out. In some cases other dilutions were employed, but in general it was found that the series given above produced good results.

The time elapsing from inoculation to the first appearance of leaf mottling varies under different conditions. The period may be as short as four days when light, temperature, and nutrition are at their optima for rapid vegetative development. Only in rare cases has the time reached 12 days, and never in the writer's experiments has it exceeded 17 days. The time required for all infected plants to develop mosaic has varied from one to nine days. During periods of cloudy weather it requires a relatively long time for symptoms to appear in all infected plants in an experiment. When growing conditions are ideal this time is usually limited to three or four days.

In the course of the experiments precautions were taken for detecting accidental infection. Since all of the inoculated plants in a given experiment do not develop mosaic simultaneously, it is not always possible to detect accidental infections on the basis of the time required for the disease to develop. The writer now makes a practice of marking a young leaf on each plant by puncturing the leaf with the inoculation needle. By this method it has been possible to determine the occurrence of mosaic on leaves of different ages on many plants. Data from over 500 plants show that no definite mosaic occurred on leaves which were over 30 mm. long at the time of inoculation. Four per cent of the plants showed mosaic on leaves which were 25 to 30 mm. long, and 95 per cent showed mosaic on leaves which were 20 to 25 mm. long, and 100 per cent showed mosaic on leaves which were under 20 mm. long at the time the plants were inoculated. When mosaic occurs on leaves which are much over 30 mm. long at the time of inoculation, and the time required for mosaic to develop is unusually short, it is considered that the plant was probably infected previous to the experimental inoculation. When mosaic does not occur on leaves which were 15 to 20 mm. long at the time of inoculation and the symptoms are late in developing on younger leaves, it is considered likely that the plant became infected accidentally after the experimental inoculations were made. It was found that the mottling was very slight on many of the leaves which were 20 to 30 mm. long when the plants were inoculated. Although the data presented serve well for the conditions under which the writer has worked, it is possible that they may not serve for all conditions of growth.

A total of 35 dilution-inoculation experiments have been conducted with fresh virus extract obtained by pressing the juice from finely chopped, diseased plants. In all experiments infection occurred in 100 per cent of the plants inoculated with fresh undiluted virus. In 31 experiments infection occurred in all plants inoculated with fresh virus extract diluted 1,000 times. In six experiments 100 per cent infection occurred in all plants inoculated with dilutions of virus up to and including 10,000 times, and in one case infection occurred on all plants inoculated with all dilutions up to and including 50,000 times.

Owing to growth irregularities which occurred in seven of the experiments, the data from these were not averaged with those of the remaining 28 experiments.

The mean data obtained from the 28 experiments are given in Table 2 and are shown graphically by the dilution-infection curve in Figure 2.

In the case of the 100 per cent mosaic datum in Table 2 the dilution datum of 5,392 times represents the mean of the highest dilutions of the virus which produced mosaic in 100 per cent of the inoculated plants. The mean dilution data were determined for the lower percentages of mosaic by averaging the dilutions at which these percentages occurred. When these data are plotted on logarithmic paper the resulting curve tends to be of the logarithmic type. The datum point for 20 per cent infection is a little low, however, in comparison with the other points.

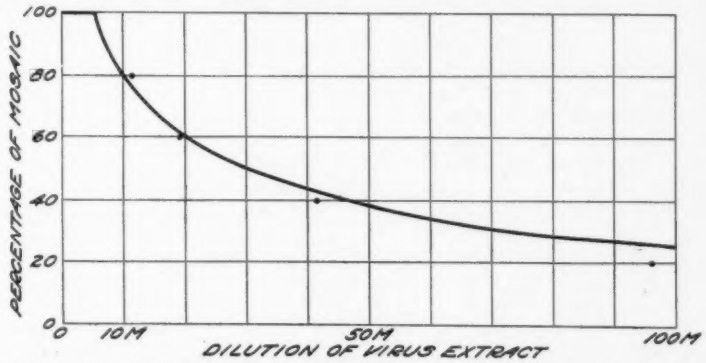

FIG. 2.—Dilution-infection curve based on the logarithmic curve of the mean data shown in Table 2. The curve is based on the average dilutions of virus at which the several percentages of mosaic occurred (Table 2)

The curve in Figure 2 was constructed on the basis of the logarithmic curve after it had been straightened. This seemed entirely allowable since the data conformed so closely to a straight line. For the present the curve in Figure 2 is being employed as a basis for the comparison of virus concentrations, and a unit of measure designated the "virus-unit" is proposed. This unit is defined as the concentration of virus in an extract which, on the average, is just capable of producing mosaic in 100 per cent of the plants inoculated according to the methods described. This concentration is designated the critical concentration. On this basis, the curve represents a concentration approximating 5,000 virus-units (5,000 V. U.) for the mean environmental conditions surrounding all of the experiments, since the mean critical concentration was reached at a dilution approximating 5,000 times.

TABLE 2.—*Means of all dilutions of virus extracts which produced mosaic in 100 per cent and in lower percentages of the plants inoculated*

Mean dilution	Percentage of mosaic
5,392	100
11,392	80
19,640	60
40,678	40
94,964	20

Figure 3 illustrates the curve in Figure 2 after it has been reduced to a single and fractional virus-unit basis. This was accomplished by dividing all of the dilution intervals given in Figure 2 by 5,000, the approximate mean critical concentration represented by Figure 2. Owing to the magnitude of this dilution, it seemed fair to neglect the fractional thousand and simplify the computations at this point. The figures on the horizontal axis of the curve in Figure 3 represent dilutions of a given volume of extract containing 1 virus-unit. The reciprocals of these dilutions represent fractions of a virus-unit. For example, at position 5 the extract has been made to five times its original volume, and, consequently, the concentration of virus in the resulting dilution equals one-fifth of a virus-unit ($\frac{1}{5}$ v.-u.).

This curve can be rather accurately expressed by the equation

$$y = 130x^{-0.27} - 30;$$ when

y = the percentage of mosaic-diseased plants obtained in a dilution test and

x = the dilution of one virus-unit.

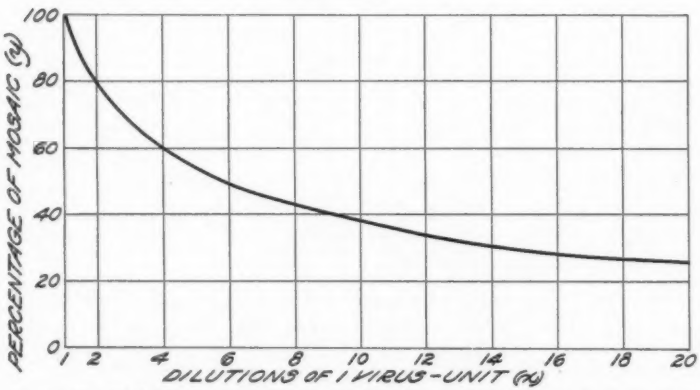

FIG. 3.—The dilution-infection curve illustrated in Figure 2 arranged on a single virus-unit (1 v.-u.) and fractional virus-unit basis. This arrangement was accomplished by dividing the dilutions (abscissae in fig. 2) by 5,000, the approximate mean critical concentration. Reciprocals of the dilution shown in this curve equal fractions of a virus-unit (1=original volume, i. e., 1 virus-unit; 2=double the original volume, i. e., ½ virus-unit, etc.). This curve is used in this paper as the standard for comparing virus concentrations

Whether this equation will hold with virus in all types of extracts is not known. It appears that it is influenced by environmental factors which influence the growth of the plant, but further study is required before these relationships will be understood. For the present it appears that the equation offers a reasonable basis for procedure, as it represents data obtained under all seasonal conditions in a greenhouse. However, it is emphasized that this position is only tentative. The summer season of 1925 at Madison was unusually cool and the sky was overcast for a greater number of days than usual. Observations at the Arlington Experiment Farm, Rosslyn, Va., also indicate that geographical locations are likely to affect the type of a dilution-infection curve to some extent. If this effect should prove to be great, it will be necessary to determine the type

of curve for the particular location where quantitative tests are conducted and to standardize all growth conditions as far as possible.

The question naturally arises as to why the number of mosaic-affected plants decreases gradually rather than abruptly as the virus is diluted beyond its critical concentration. At the present time it seems reasonable to explain this relationship on a chance basis. Regardless of the nature of the virus, its particles become more widely separated as the extract is diluted, and, accordingly, the chance that the required number of particles will be so placed in the plant as to produce mosaic becomes less as the dilutions increase. If the virus particles are adsorbed on colloidal or on larger particles in the extract, it seems probable that any change which may cause a release of the virus particles will increase the chances for producing mosaic.

The dilution-infection curve indicates that there is a marked acceleration in activity on the part of the virus after the dilution passes beyond the point of the critical concentration. Although these curves resemble a curve representing the actual reduction in concentration of any known substance which is being diluted, it is evident that the physiological activity of the virus as measured by mosaic symptoms is in excess proportion to the actual relative concentration of the virus in the diluted samples of extract. This accelerating effect may be due to conditions arising in the dilution samples or to conditions in the test plants. There is some evidence that the dilution-infection curve may rise slightly when the dilution of the virus extract is carried to 200,000 to 400,000 times, but this point can be tested in a more satisfactory manner after the completion of other studies.

The dilution-inoculation method may be applied in several ways, depending on the nature of the problem under investigation. When the investigator is not familiar with a virus extract it is very difficult to select at random those dilutions which will contain the critical concentration of the virus. In this case it is best to make several dilutions of the extract and to inoculate five or more plants with each. For this work the writer usually uses the extract undiluted and diluted 1,000, 10,000, 50,000, and 100,000 times in sterile distilled water. Further tests can then be made in the region near the critical concentration by employing several dilutions which may enable a more accurate location of this concentration.

The tests also may be carried out in the region between the critical concentration and the point of 40 per cent infection. When this method is employed the writer uses but one or two dilutions of the virus extract and from 10 to 30 test plants for each dilution sample.

After having determined the percentage of mosaic-diseased plants produced by an unknown diluted extract (y in the equation for the infection curve), the virus-unit concentration is computed according to the following equation.

$$\text{V. U.} = \frac{x'}{x} \text{ when}$$

x' = dilution of unknown extract
x = dilution of 1 virus-unit.

Example: If the experiment shows that an unknown extract diluted 10,000 times produces mosaic in 60 per cent of the plants inoculated, then $x' = 10{,}000$ and $y = 60$. From the plotted curve

(fig. 3) it is then found that when $y=60$, one V. U. has been diluted four times, or $x=4$.

By carrying out the computation it is found that the

$$\text{V. U.} = \frac{10{,}000}{4} = 2{,}500,$$

that is, the virus concentration of the unknown sample approximates 2,500 virus-units for one-eighth c. c. of extract, the approximate volume used to inoculate each plant.

When several dilutions of the same virus extract are tested simultaneously the writer computes the virus-units for each dilution test beyond the point of 100 per cent infection and not including 0 per cent infections at the end of the series. All of the virus-unit determinations are then averaged, and this average is taken as the approximate virus concentration of one-eighth c. c. of the extract being tested.

As dilution-infection curves have approached 20 per cent to 0 per cent infection, experimental variation seemed to increase, and it has not been uncommon for infection to reach 0 at a given dilution and then rise a little at the next higher dilution. In this case the 0 per cent infection was averaged with the virus-unit calculations for the remaining dilutions. Sometimes the curve drops and rises again with the higher percentages of infection, especially when the dilution steps are close together. However, this has occurred in but few cases in which the populations were small.

In some cases, as in the experiments cited in Tables 4 and 5, the writer did not select dilutions sufficiently low to give the best infection data. In Table 4 it will be noted that in certain cases no infection occurred in any of the plants inoculated with extract diluted above two times. In such cases it is not possible to compute the virus-unit concentration of the fractions. However, from the data in Table 4 we know that the filtrate fractions did contain virus, because mosaic occurred in 100 per cent of the plants which were inoculated with extract diluted to twice its original volume. From the data in Table 5 there is no way of determining if virus was present in the filtrate fraction. As it is certain that these filtrates contained less virus than the unfiltered extracts, it is possible to determine the approximate highest value for the virus-units in them. In this case the writer assumes that if one more test plant had been inoculated with the filtrates diluted 1,000 and 100 times, respectively, it might have in each case developed mosaic. Thus, 24 virus-units in Table 4 and 1.3 virus-units in Table 5 represent the highest possible values for the fractions, and it seems likely that the values were actually less than these.

From the experiments carried out thus far, it is believed that the most accurate results can be obtained by determining the position of the critical concentration or by basing the virus concentrations on infection percentages which are close to the critical concentration. However, as stated previously, it is rather difficult, in most cases, to estimate the position of the critical concentration for purposes of selecting the dilution intervals which will yield data in the desired region of the curve. As a result, most of the writer's data include all or nearly all of the infection range of the dilution-infection curve.

When more is known of the relative influence of the growth factors on the concentration of virus in plants it will be easier to estimate the dilutions which will yield the most accurate data.

It may be suggested that, owing to the variables which are not under control, there is no justification for endeavoring to use a virus-unit system. The writer took this position for some time, and expressed the experimental results as percentages of the total number of plants inoculated. However, it was soon found that this method gave no idea as to numerical proportions between concentrations. For example, if a given fraction produced mosaic in 80 per cent of the plants inoculated, and the same dilution of another fraction caused mosaic in 40 per cent of the plants, it could not be concluded that the first fraction had double the virus concentration of the second fraction. This is evident from the type of infection curves shown in Figures 2 and 3. As a matter of fact, the first fraction is about five times more concentrated than the second. It seemed advisable, therefore, to devise a method which would express proportions with greater exactness, and, while several possibilities presented themselves, the virus-unit method appeared reasonably satisfactory for the present.

From the results obtained thus far, it is evident that experiments of a similar nature should be repeated several times. Large populations of test plants should be employed when possible. To this end, the number of dilutions should be reduced as much as possible in order to facilitate the use of larger numbers of plants for a dilution test. In this way experimental variation may be reduced to the minimum.

PURIFICATION STUDIES

FILTRATION

Of the physical methods of separation, filtration probably has been used more generally than any other for purifying many biological compounds. In studies on the virus of mosaic it has been demonstrated many times that the virus passes certain filters and, consequently, the filtered virus is partially purified through the removal of the larger suspended and colloidal materials which were present in the original extract. It has long been known that ordinary charcoal and sand filters remove more of the impurities from water after a considerable amount of débris or slime has collected in the filters than they did before. This relationship is known also to hold generally in commercial filtration processes, and it has been recognized by those who produce various biological products, such as serums, vaccines, and antitoxins, that filter slime may itself be a good filter, depending on its nature (*13*).

In the ordinary filtration procedures with paper filters it is evident that a filtrate of extract from freshly collected plant leaves becomes decidedly clearer as filtration proceeds. A simple experiment conducted with soft filter paper, an ordinary ribbed, glass funnel, and 400 c. c. of freshly expressed juice from tobacco leaves showed that the first 10 c. c. of filtrate contained sufficient chlorophyll to render it almost as green as the unfiltered juice. The second 10 c. c. contained very little chlorophyll, but there was much brown-colored material in suspension; so much, in fact, that light from a 100-watt mazda lamp with a concentrated filament would not pass through the filtrate when in a test tube 16 mm. in diameter. In the case of the

fourth 10-c. c. sample of filtrate, the light passed through slightly, and in each succeeding sample it passed more readily, until in the fourteenth 10-c. c. sample, the filtrate was very clear, and the light shone through with great brilliance. When all of the samples of filtrate had been heated in a steamer long enough to bring about coagulation of part of the proteins, it was found that the amount of coagulum gradually decreased from the first to the fourteenth sample.

With this evidence in mind, it appeared reasonable to believe that the slime which collects on the surface of biological filter candles does act as a filter, thus rendering it difficult to determine the actual filtration capacity of a given candle for the virus of mosaic.

To test this point with a virus, the writer set up an experiment in which ordinary, soft-paper filters were employed in Büchner funnels 4 inches in diameter. Fresh, undiluted juice from mosaic-affected tobacco plants was heated at 65° C. for 70 minutes in order to coagulate the suspended material and a portion of the heat-coagulable proteins. This heating does not seem to impair the potency of the virus, and the resulting coagulum makes possible the use of paper filters for clarifying small volumes of fluid.

After the coagulation process, two 15 c. c. samples of the fluid were put in two Büchner funnels and the filtrates were obtained under a vacuum of 30 inches of mercury. The resulting filtrates were very clear. One of these, and also its filter slime, were tested for virus concentration. The other filtrate was discarded, but its slime was carefully washed on the filter with 150 c. c. of cold, distilled water, after which it was made up to the original volume (15 c. c.) and tested for virus concentration. The results of these experiments are shown in Table 3.

TABLE 3.—*Virus concentrations in unfiltered fresh virus extract; in unfiltered fresh extract, heated at 65° C. for 70 minutes and filtered through soft filter paper; and in filter-slime precipitate, unwashed and washed*

Materials	Percentage of mosaic at dilutions of [a]—							Average virus-units
	0	100	1,000	5,000	10,000	50,000	100,000	
Unfiltered extract	100	100	100	80	60	20	0	2,222
Filtrate	100	100	40	0	0	0	0	111
Precipitate	100	100	100	80	60	20	0	2,222
Washed precipitate	100	100	100	60	0	20	0	988

[a] Five plants inoculated with each dilution.

From these data it is evident that filter slime, under certain circumstances at least, does remove a considerable quantity of the virus. The results from the washed precipitate seem to indicate that a considerable portion of the virus was held by the slime in a physical rather than a chemical manner.

Further tests made with filtrates from fresh, unheated juices, were low in virus concentration as shown in Tables 4, 5, and 6. In Table 6 it will be seen that the removal of part of the slime by means of the centrifuge previous to filtration seemed to enable a greater quantity of virus to pass the filter than when all of the slime was present.

Further tests on the filtration of substances have shown that the presence of filter slime offers considerable difficulty, and it now appears that, under certain conditions, the colloidal and other suspended materials which make up the slime are effective filters.

TABLE 4.—*Virus concentrations of unheated fresh virus extract, diluted with an equal quantity of distilled water; unfiltered and filtered*

Materials	Percentage of mosaic at dilutions of [a]—					Average virus-units
	2	1,000	10,000	50,000	100,000	
Unfiltered extract	100	100	60	40	0	4,027
Berkefeld V filtrate [b]	100	0	0	0	0	[c] 24
Berkefeld V filtrate [b]	100	0	0	0	0	[c] 24
Berkefeld W filtrate [b]	100	0	0	0	0	[c] 24

[a] Five plants were inoculated with each dilution.
[b] Two tests were made on the "V" filtrate and one test was made by passing the "V" filtrate through a "W" candle.
[c] This value is based on the possibility that one out of six plants inoculated with extract diluted 1,000 times might have developed mosaic. See explanation in text.

TABLE 5.—*Virus concentrations of unheated undiluted virus extract; unfiltered and filtered*

(The extract stood in the laboratory for five hours before the filtration was made)

Materials	Percentage of mosaic at dilutions of [a]—			Average virus-units
	100	1,000	10,000	
Unfiltered extract	100	100	90	7,692
Berkefeld V filtrate	0	0	0	[b] 1.3

[a] Ten plants were inoculated with each dilution.
[b] This value is based on the possibility that 1 out of 11 plants inoculated with extract diluted 100 times might have developed mosaic. See explanation in text.

TABLE 6.—*Virus concentration of fresh undiluted virus extract; of a portion of the same extract after it had been centrifuged for seven minutes at approximately 50,000 revolutions per minute, and of filtrates from fresh and from centrifuged extract*

Materials	Percentage of mosaic at dilutions of [a]—						Average virus-units
	0	500	1,000	10,000	50,000	100,000	
Fresh extract	100	----	100	80	0	0	5,000
Centrifuged extract	100	----	100	40	0	0	1,111
Filtrate, fresh extract [b]	100	20	20	0	0	----	25
Filtrate, centrifuged extract [b]	100	60	80	20	0	----	317

[a] Five plants were inoculated with each dilution.
[b] A Berkefeld V filter candle was used.

By using hemoglobin as a test substance, it was found in two experiments that approximately 18 and 50 per cent of the hemoglobin in 1 per cent water solutions passed a Berkefeld V filter (⅝ by 1½ inches) at the rate of 10 c. c. in 45 and 15 seconds, respectively. The reduced concentration of hemoglobin in the first filtrate was due to extraneous material which collected on the filter and formed a slight

slime. This material was removed from the hemoglobin by means of a supercentrifuge before the second experiment was started. A 1 per cent hemoglobin solution was then prepared by mixing a 2 per cent water solution of hemoglobin with an equal quantity of fresh, undiluted, unfiltered tobacco fluid from very young plants.[4] This solution was filtered in the same filter candle referred to above, after it had been thoroughly cleaned. No visible hemoglobin passed with the filtrate. In fact, the presence of the hemoglobin in the unfiltered extract so improved the filtering properties of the slime deposit that about one-half of the lemon-yellow pigment in the fluid was removed when the filtrate came through the candle.

Three hours were required for 7 c. c. of this filtrate to pass the filter. By carefully washing the filter it was found that no visible hemoglobin got beyond the filter slime. This whole experiment was repeated and the same results were obtained.

Although the filtration experiments are preliminary, the results seem sufficiently consistent to warrant the general conclusion that filtrates are relatively low in virus concentration. The filtrate concentrations, as found in these experiments, ranged from about 20 to 5,900 times less than the concentrations of the unfiltered extracts. It is apparent that certain of the suspended materials in an extract constitute a source of error and difficulty in filtration studies, and it appears that unpurified virus can not be employed with certainty for studies on the determination of the size of the virus particles by means of filters (*5*).

This interpretation is borne out by results of colloid chemists who have studied particle sizes by means of filters. Even with purified colloids it is found that filters offer many difficulties, some of which may be reduced through the use of the supercentrifuge (*23*).

In some cases the variability of filtration of a given substance has been explained on the basis of differences in the electrical charge on the filter. This explanation is doubtless correct for substances which have been purified to a considerable extent. However, in the case of complex plant extracts and animal sera it is likely that the effect of an electrical charge is sometimes more indirect than direct. The physical arrangement of the suspended materials in plant extract can be greatly altered by changing the electrical charge. Thus, the arrangement of these materials (slime) on the filter will alter the porosity of the slime layer and thereby alter the passage of virus.

GRAVITATION AND SUPERCENTRIFUGATION

As the quantitative virus studies indicated that the filtration process offers several difficulties when it is desired to recover highly concentrated virus, it seemed desirable to explore the possibilities of using gravity and centrifugal force to effect the desired separations. By these methods it is possible to recover all fractions more completely than by the use of filters. Moreover these methods do not introduce the difficulties resulting from the slime layer which forms on filters.

A preliminary experiment was carried out with fluid from tobacco plants affected with mosaic. This fluid was allowed to stand in a flask in a refrigerator for 12 hours. At the end of this period the re-

[4] The filtered juice from these young tobacco plants was colored a faint lemon yellow, and small traces of hemoglobin could be detected in it quite readily.

sulting supernatant fluid was decanted into another container. At the end of another 12 hours a second supernatant fluid was evident in the container which held the original sediment. At this time samples of the first and second supernatant fluids and of the final sediment were tested for virus concentration. As indicated in Table 7, the greatest virus concentration occurred in the supernatant fluid next above the sediment.

TABLE 7.—*Virus concentrations of three gravity fractions of virus extract which had been diluted with an equal volume of distilled water and allowed to stand in a refrigerator for 12 hours*

Fractions	Percentage of mosaic at dilutions of [a]—						Average virus-units
	2	2,000	10,000	20,000	100,000	200,000	
1 (upper)	100	100	100	80	40	20	9,259
2 (intermediate)	100	100	100	80	80	60	36,666
3 (bottom)	100	100	40	60	0	0	3,055

[a] Five plants were inoculated with each dilution.

The results of this experiment indicated that much of the extraneous material in fluids might be removed by centrifugation without reducing the virus concentrations as rapidly as by filtration. Preliminary tests with ordinary types of test-tube centrifuges soon indicated that the speeds were too low to effect good separations of fresh fluids. It was found that separations were much more easily made in fluids which had been heated at 65° C. or allowed to stand in the laboratory long enough to effect coagulation of a portion of the suspended materials. However, it was soon apparent that the juices from different lots of plants of the same species varied considerably. Some would not show coagulation on heating at 65° or on standing at room temperature for long periods. For this reason, experiments were carried out with high-speed centrifuges.

The Sharpless supercentrifuge was first employed, and it was found that much of the suspended material in the most refractory extracts could be removed in a few minutes when the fluids were rotated at 40,000 revolutions per minute. The laboratory model of this centrifuge offers several possibilities, but it was found to be too large for studying small volumes of fluid.

Search for a centrifuge revealed one which, after some special adaptations, served admirably for these studies. This machine is an outgrowth of several centrifuges developed by E. A. Birge and C. Juday for their studies on the Wisconsin lake plankton. It had been described by Juday (*12*) and is again described in this paper in order that certain modifications may be noted (figs. 4 and 5).

The machine consists of a Dumore No. 3 multispeed grinding motor and spindle mounted vertically on a right-angle cast-iron base. The centrifuge cup is screwed on the upper end of the spindle.

For centrifuging a continuous flow of fluid the cup is mounted in a brass housing on the top of the spindle casting. For the study of small batches of fluid this housing is not necessary.

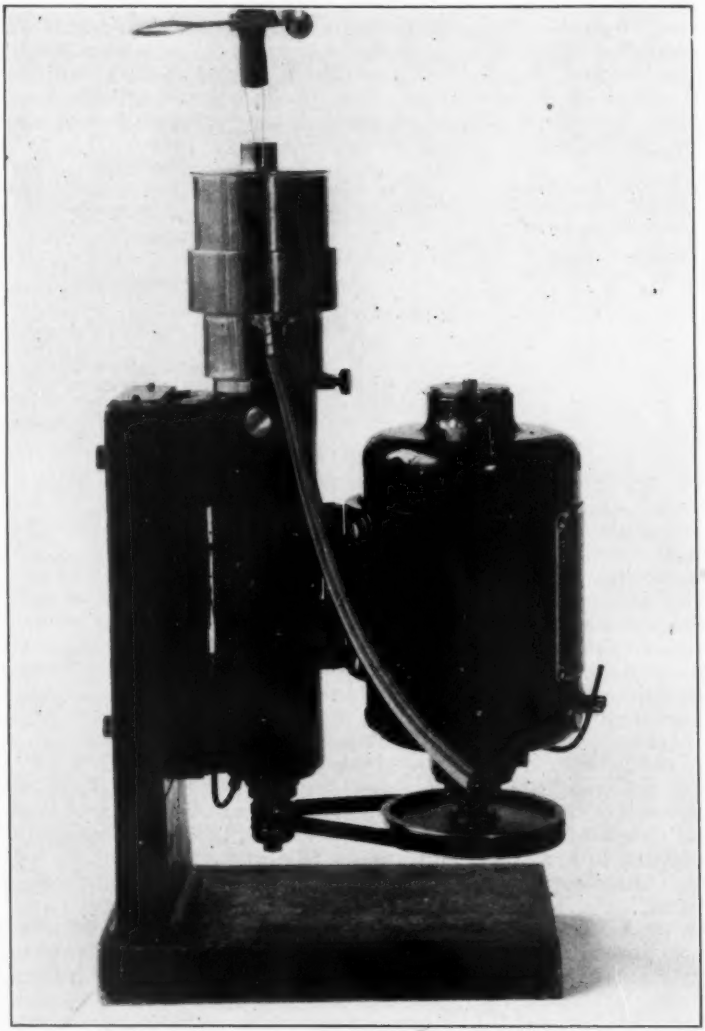

FIG. 4.—Supercentrifuge, constructed by attaching a high-speed motor and grinding spindle to a cast-iron upright. The centrifuge bowl is screwed on the top end of the spindle, which is inclosed in the housing and water jacket shown in Figure 5. As illustrated, the machine is arranged for centrifuging a continuous flow of fluid entering at the top. The solid material remains in the bowl and the liquid fraction runs out through the lateral rubber tube

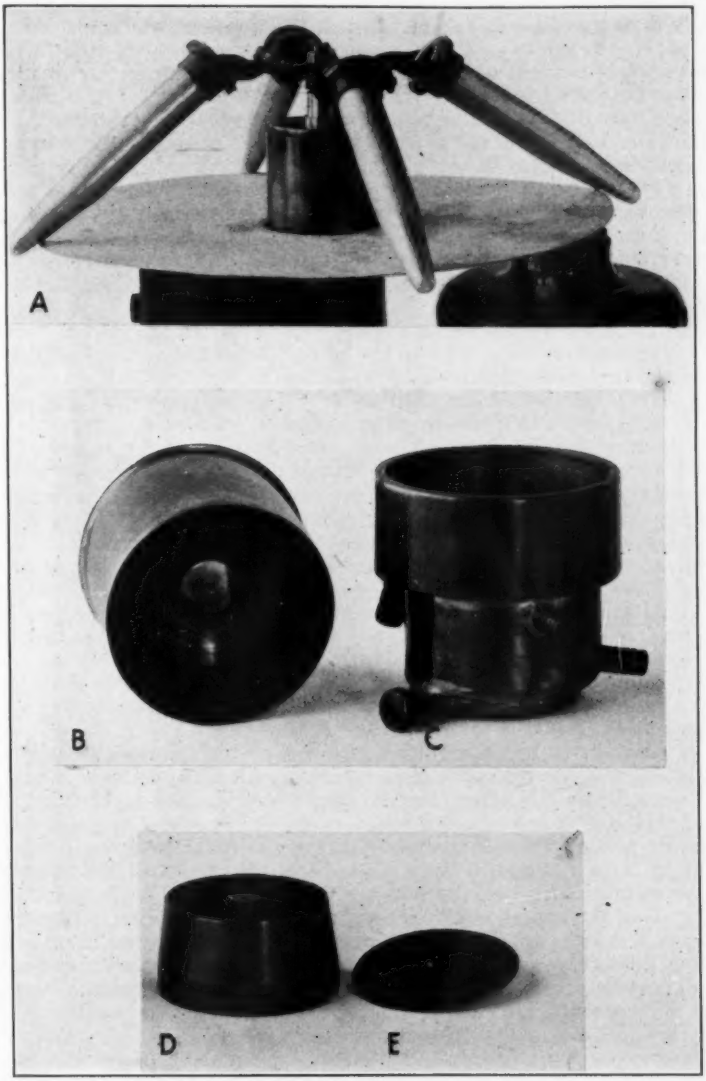

FIG. 5.—Parts employed in connection with the centrifuge shown in Figure 4: A, an ordinary sedimentation tube head which can be put on the spindle in place of the bowl; the metal disk prevents the shields from striking the motor and support; B, the housing cover, which is used when centrifuging a continuous flow of fluid; this cover fits into C, and its internal disk fits into the bowl D to prevent the liquid from flying out of the bowl before it has been subjected to centrifugal force; C, the housing which serves as a collecting head for the centrifuged fluid that comes over the upper edge of bowl D, and also as a water jacket for cooling the upper bearings of the spindle; D, the bowl; this rotates in B and C when a continuous flow of fluid is being centrifuged; E, a thin spring-brass cover which is screwed to the central post in cup D, when small quantities (4 to 8 c. c.) of fluid are centrifuged; in this case the housing cover B is not used

In most of the studies single small lots of fluid, ranging from 4 to 10 c. c. were employed. As the centrifuge is run at its maximum speed, approximately 50,000 revolutions per minute, it is obvious that a great amount of evaporation takes place in an open cup such as was employed by Juday. For this reason the writer now uses a bronze bowl 2 inches in diamater and 1 inch deep (inside dimensions). The walls form an acute angle of 8° with the bottom. By turning a small bead on the inside of the top edge the bowl holds approximately 8 c. c. of fluid when running at full speed. The central post in the bowl is slightly lower than the edges of the wall. A small hole in the top of this is tapped to take a small screw, which fastens a very thin, spring-brass cover over the bowl. This cover prevents evaporation and thus enables accurate tests to be made on the different fractions.

Earlier, a bowl with vertical sides was constructed, with a cover which screwed onto the walls of the bowl. After some experimenting, it was found almost impossible to obtain a sufficiently tight cover to prevent the loss of the fluid while it was being centrifuged, and as a consequence this construction had to be discarded.

In certain studies it was necessary to centrifuge for a rather long time, and frequently the machine was in use over long periods while several successive samples were being centrifuged for short intervals. Under these conditions the upper bearings of the spindle got very warm and the speed was sometimes reduced on this account. To eliminate this difficulty the writer designed a water jacket at the base of the bowl housing, as shown in Figure 5, C.

To oil the bearings small copper pipes were inserted in the oil holes at the bottom bearings of the motor and spindle. These were turned upward so that the oil might be introduced readily. It is estimated that this machine exerts a force almost 70,000 times greater than the force of gravity when rotating a bowl 2 inches in diameter at approximately 50,000 revolutions per minute.

In addition to the mechanical problems which arose, many new problems were encountered in connection with plant fluids and with the virus. For this reason the data presented can not be considered final. However, in general, they are so consistent that it seems worth while to present them in summarized form in order to throw further light on the virus and to show more clearly the value of the quantitative method which has been described.

In all of the experiments carried out the solid fractions were carefully removed in a volume of sterile distilled water equal in weight to the fiuid fraction removed. Therefore, the virus concentrations in all fractions were compared on practically an equal-volume basis. The differences in the specific gravity of the fluids were so small that they are ignored in the present discussion.

Four experiments were conducted to determine the concentration of virus in the heavy chlorophyll-bearing material present in fresh extract from diseased plants. This fraction usually can be separated from 4 c. c. of fluid in about 15 seconds by centrifuging at full speed. From results of these experiments, shown in Table 8, it is evident that this first heavy fraction is very low in virus content as compared with the remaining fluid fraction.

TABLE 8.—*Virus concentrations in solid and fluid fractions from a fresh virus extract centrifuged in a closed bowl for one minute at approximately 50,000 revolutions per minute*

Experiment No.	Fraction	Percentage of mosaic at dilutions of[a]—					Average virus units
		0	1,000	10,000	50,000	100,000	
1	Control (uncentrifuged extract)	100	100	60	20	20	2,499
2	do	100	100	40	20	20	2,036
1	Solid	100	40	0	0	0	111
2	do	100	40	0	0	0	111
3	do	100	20	0	0	0	33
4	do	100	80	0	0	0	500
Mean							188
1	Fluid	100	100	40	20	20	2,036
2	do	100	100	20	20	0	1,000
3	do	100	100	80	60	20	6,944
4	do	100	100	80	20	20	3,333
Mean							3,328

[a] Five plants were inoculated with each dilution.

An experiment was carried out to determine if centrifuging the extract for longer periods would cause the virus concentration in the solid fraction to increase. It was found that the concentration did increase in the solid fraction when the fluid was centrifuged for nine minutes, but the increase did not seem to be very great.

It was then decided to determine the effect of combining heat treatment with long centrifuging. The results of this experiment are given in Table 9. It will be seen that this combination makes possible the removal of a large percentage of the virus from the fluid fraction.

Eight additional experiments were conducted on this phase of the problem. The data are given in Table 10. In each of these experiments it was found that the virus concentration in the solid fraction from whole extracts which were centrifuged for short intervals (one-half to one minute) was low, and that the virus concentration in the fluid fraction was high. However, by heating the extracts at 65° C. until coagulation occurred and then centrifuging them for 5 to 10 minutes, it was possible to remove a large percentage of the virus from the fluid and concentrate it in the solid fractions.

In all of the centrifugation studies thus far conducted it appears that the virus concentration of the fluid fraction can be reduced most readily in extracts which have been heated at 65° C. for 10 to 15 minutes, or which have stood in the laboratory for several hours.

In general, it seems evident that the results of the gravitation and the centrifugation experiments agree in that the virus concentration is greatest in what might be termed the "intermediate" fraction. Whether the small amount of virus which remains in the fluid fraction after long centrifugation is the result of contamination from the solid fraction, or whether it represents a form which is in solution, is not known. When the centrifuge stops there is considerable washing of the solid fraction by the liquid fraction, and a portion of the virus which has been deposited may be taken up again by the fluid. The ultimate solution of this problem depends to some extent on further improvement of the apparatus.

TABLE 9.—*Virus concentrations in solid and fluid fractions from fresh virus extract centrifuged for 2 seconds; in solid and fluid fractions from virus extract which, after having stood for 4 hours at room temperature, was heated at 65° C. for 15 minutes and centrifuged for 10¼ minutes at approximately 50,000 revolutions per minute*

Fractions	Percentage of mosaic at dilutions of [a]—			Average virus-units
	100	1,000	10,000	
Solid fraction from fresh extract centrifuged for 2 seconds [b]	90	40	20	174
Fluid fraction from fresh extract centrifuged for 2 seconds	100	100	100	10,000
Solid fraction from 4-hour extract, heated at 65° C. for 15 minutes and centrifuged for 10¼ minutes [c]	100	100	80	5,000
Fluid fraction from 4-hour extract, heated at 65° C. for 15 min- and centrifuged for 10¼ minutes	90	50	10	128

[a] Ten plants were inoculated with each dilution.

[b] It was found that practically all of the chlorophyll-bearing material in this particular sample of extract was removed from the fluid by centrifuging for the unusually short time of 2 seconds at full speed.

[c] This fraction was obtained by centrifuging the sample in two operations, thus causing some loss of virus However, it is believed that the difference between the concentrations of virus in the fluid from the sample centrifuged for 2 seconds and of that in the solid fraction from the sample centrifuged for 10¼ minutes is largely due to experimental variation which would have been reduced if a larger number of test plants had been used.

TABLE 10.—*Mean data from eight experiments, showing the virus concentration in solid and fluid fractions from extracts centrifuged for short periods (one-half to 1 minute) without heating, and for long periods (5 to 10 minutes) after heating at 65° C.*

Time of centrifuging	Fraction	Mean percentage of mosaic at dilutions of—							Average virus-units
		0	100	1,000	10,000	25,000	50,000	100,000	
Short time	Fluid	100	100	90	50	30	20	20	1,842
	Solid	100	70	43	10	6.6	0	0	142
Long time	Fluid	100	73.3	48	32	6.6	0	0	307
	Solid	100	100	90	50	40	0	0	1,755

STUDIES ON THE VARIABILITY OF VIRUS EXTRACTS

The quantitative method has been applied to several virus problems other than those cited, but there are fewer data on these. However, it may be stated that evidence now available indicates that virus concentrations in expressed extracts are influenced considerably by the method of extraction. In one experiment the extract from fresh mosaic-affected tobacco leaves, passed through the fine cutter of a food-chopper, had a virus concentration of 6,332 virus-units. When the press cake or tissue from which this extract was removed was ground with quartz sand in a quantity of water equal to the quantity of extract removed the resulting expressed extract contained 5,479 virus-units.

In a second similar experiment it was found that the first extract contained 6,667 virus-units, and the second extract, obtained after grinding the press cake with sand, contained virus amounting to 3,333 virus-units. A hand press was used in all of the experiments reported. Had a hydraulic press been available, it is probable that the virus concentrations of extracts from tissue passed through a food chopper would have been greatly increased. Doubtless the crushing of the cells with sand aids in increasing the yield of virus.

However, it appears that the presence of the sand in the press cake aids the virus in escaping from the press cake into the press fluid. Experiments on frozen tissue seem to bear out this point to some extent.

Virus concentration also varies in different parts of the same plant. In one experiment it was found that extract from the six uppermost leaves of a mosaic-mottled tobacco plant contained virus amounting to 3,072 virus-units. The extract from the stem to which these leaves were attached contained only 33 virus-units. In a second similar experiment the leaf extract contained 38,461 virus-units and the stem extract 3,255.

It has been found that the virus concentration is from two to four times greater in succulent plants than in older woody plants. The upper mottled leaves of a diseased plant contained about one-third more virus than the lower unmottled leaves of the same plant.

Extracts from the tobacco plant vary greatly in their ability to produce a marked coagulum and precipitate on standing at room temperature or on being heated. In some cases a marked coagulum will develop and settle out of expressed tobacco fluid when it has stood for two or three hours, whereas fluid from tobacco plants grown under different conditions may show no change to the eye after standing three or four days. Under certain conditions, a good coagulum will develop and settle out of freshly expressed tobacco juice when heated 5 or 10 minutes at 40° C. yet it is not uncommon to find tobacco extract which shows no apparent coagulation until the temperature reaches 90° or above. All gradations of coagulation have been observed between these temperatures. On further study with supercentrifuged extracts it was found that some of the protein and other substances do coagulate in all of these fluids when heated. However, under certain conditions this is masked by the chlorophyll-bearing material, which does not always coagulate and settle out at lower temperatures. In general it may be said that the extracts from tobacco plants grown in the summer coagulate more readily than do those from plants grown in the autumn, winter, or spring.

Juices from all plants of the same species do not filter alike. In some cases it is possible to obtain complete filtration of a given quantity of fresh extract in from 10 to 15 minutes, whereas an equal quantity of another lot of fluid similarly handled may require 3 to 4 hours to pass the same filter candle. In the centrifugation studies it was found that some fluids would clarify in 5 seconds, whereas others required almost 60 seconds to reach the same degree of clarification. Extracts from summer-grown tobacco plants seem to filter and clarify more readily than do those from plants grown in the autumn, winter, and spring. Extracts from old plants are less easily filtered and clarified in the centrifuge than are the extracts from younger plants. Several tests have shown that extracts from old plants do not produce a coagulum as readily on heating as do the extracts from young plants. The age of plants seems also to influence the rate of precipitation of a coagulum produced by heat or by standing at room temperature. The conditions that regulate the growth processes—as nutrition, temperature, soil, atmospheric moisture, and light—seem also to regulate the behavior of plant extracts in vitro. It would therefore appear that if uniform extracts are to be obtained

for use in experiments of the sort described, more attention must be given to the regulation of the growth conditions and to the selection of the plants employed.

In addition to the variables which are unavoidable in the culture of the plant there are others which are due to the methods employed in handling the extracts. In general it has been found that extracts filter and also clarify in the centrifuge more readily after they have stood in the laboratory for some time, been frozen, treated with certain chemicals, or after they have been heated at 65° C. for 5 to 10 minutes. It is apparent that anything which changes the physical or chemical state of the suspended material in an extract is likely to affect filtration and clarification.

CONCLUSIONS

Experiments which are presented in this paper indicate that the virus of tobacco mosaic can be studied quantitatively through the use of dilution and inoculation methods. The method which is suggested has been tested in connection with the study of several problems, and the relative congruity of the virus concentrations in fractions obtained in a similar manner lends support to the method.

The accuracy of the method depends on several factors which are not easily controlled. In order to reduce experimental variation to a minimum, it is necessary to use large areas of greenhouse space for the many test plants. This makes it difficult to control temperatures as accurately as would be possible in small chambers. At the present time it is impossible to obtain even approximately uniform sunlight conditions for experiments carried on at different periods of the year. However, in spite of these variables, it seems evident that the method offers a basis for the further improvement of quantitative methods.

The preparation of virus in some standard form which may be employed as a control in quantitative tests of virus of unknown concentration should tend to simplify the present difficulty of growth variables which occur in experiments that are not conducted simultaneously. A similar procedure has made it possible to perfect the biological assay of certain drugs and antitoxins.

Data presented show that filtrates of virus extract tend to be low in virus concentration. These data indicate that the filter slime, which is composed of suspensoidal and colloidal materials contained in the extracts, removes a considerable amount of the virus. On this account it is impracticable to employ filters in certain stages of purifying a virus and in making studies on the size of the virus particle.

Tests carried out with a supercentrifuge show that the heavy suspended particles in a virus extract can be removed by centrifugal force without seriously reducing the concentration of the virus in the fluid fraction. A large proportion of the virus can then be removed from the fluid by heat coagulation and further centrifugation.

Preliminary experiments indicate that the concentration of the virus varies in plants grown under different conditions, in plants of different ages, and in different parts of the same plant. It was also found that the concentration of virus in an extract is influenced by the methods used in preparing the tissue and in making the extraction. The chemical and physical behavior of extracts also varies in plants

of different ages and in plants of the same age but which are grown under different conditions.

From the results presented, it is evident that the methods of culturing and selecting the plant material, and the methods employed in making and manipulating the virus extracts must be standardized just as far as possible to insure reasonably uniform results.

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AGE OF POLLEN AND OTHER FACTORS AFFECTING MENDELIAN RATIOS IN MAIZE[1]

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INTRODUCTION

Mendelian inheritance is based upon the assumption that the heritable elements or genes derived from the parents remain unchanged and are distributed equally to the gametes. Under normal conditions, therefore, the gametes of hybrids should fall into two equal classes with respect to any given character pair. That such is indeed the fact is attested by innumerable experiments. With some characters, it is true, orthodox ratios are not always found. Most of these cases, however, can be explained by a differential viability of the zygotes or other causes not associated with the ratios of the genes in the gametes.

The present thesis is that modification of the Mendelian ratios can occur in the gametes. The genes here dealt with are the *C c* pair involved in the production of aleurone color in the seeds of maize, and the *Wx wx* pair that determine whether the seeds shall be horny or waxy.

Both pairs have been used in numerous experiments in the past decade and the *C c* pair has always behaved in orthodox fashion. The *Wx wx* pair, on the other hand, though closely approximating a simple Mendelian behavior, has given, with some consistency, a deficiency of *wx wx* zygotes of 1 or 2 per cent.

Several explanations have been suggested to account for this slight deficiency but it now appears that these are inadequate, for not only are the *wx wx* zygotes often below the expected but under certain circumstances they are greatly in excess of the expected proportions.

From experiments with the storage of pollen it seems clear that in fresh pollen the effective gametes bearing the *wx* genes are below equality, whereas in old pollen such gametes are in excess of equality. The hitherto observed approximation of the percentage of waxy seeds to the Mendelian expected seems to have been the result of a fortuitous blending of fresh and old pollen, or to the even more remarkable coincidence—the use of pollen of just that age where the two sorts of gametes function equally well. In a certain sense the alteration in the proportion of the *Wx wx* genes in the stored gametes may be considered as selection, differentiating between gametes.

Although a legitimate difference of opinion exists as to the rôle of natural selection in evolution, there can be no question that stringent conditions result in the elimination of maladjusted organisms. Certain combinations of hereditary factors doom the individual possessing them to an early death, and still others make survival

[1] Received for publication Jan. 22, 1927; issued July, 1927.

difficult except under the most favorable conditions. With plants the operation of natural selection is an ever-present and clearly visible phenomenon. This is well illustrated by those plants that inherit a genetic combination which prevents the development of chlorophyll. Geneticists are familiar with numerous heritable conditions that effect the vitality of the organism; some as in albinism, causing death at very early stages and others permitting the plants to struggle through to the production of a few seeds. Practically all mutations have a lower vitality than the normal forms from which they came, and are, therefore, more susceptible to adverse environment. Such cases illustrate natural selection operating upon zygotes.

From the standpoint of greatest economy of effort, a selection operating upon gametes offers many advantages. Especially is this true of plants, where millions of gametes are formed and exposed to external conditions for every one that functions successfully.

If external factors are to differentiate between gametes it is necessary that the characteristics of gametes be influenced by the genes they bear. The opportunity to measure directly any influence of the genes on the character of the gametes is limited. However, if it were found that adverse conditions operated as a selective agent in such a manner as to alter Mendelian proportions, it would be a fair inference that the genes had affected the characteristics of the gametes.

Not many examples, however, have been reported of selection operating upon gametes. It is true that selective fertilization is a well-established phenomenon and that certain classes of self and cross sterility involve gametic selection. These cases, however, all concern the interaction of the genetic factors of the gametes of opposite sexes and properly can not be considered as selective differentiation in the sense of external factors affecting unequally the different sorts of gametes.

Belling (*1*)[2] and others have shown that under normal conditions certain genetic factors are lethal to the gametes carrying them, but it is possible that under more favorable circumstances some of these gametes could effect fertilization. If this were possible such cases would provide an example of an external force operating as a selective agent upon gametes. However, no clearly authenticated examples of the revival of genetically sterile gametes, by unusually favorable conditions, have been reported and these cases must be left in the realm of possibility only.

In animals it has been shown that alcohol, when administered to prospective parents, acts as a selective agent not only on the newly constituted zygotes but on the gametes as well (*12*). The experiments with alcoholized animals furnish the best authenticated examples of selection operating upon the gametes. However, no evidence has been presented to show that specific genes ultimately determine the fate of the gamete bearing them. The evidence for selection rests upon such multiple gene characters as size and vigor as expressed by the offspring of alcoholized parents and the selective agent apparently operates upon a combination of genes.

With most genes their presence in the gamete can be inferred only from breeding tests, but in the case of the gene for waxy endosperm in the seeds of maize its presence in the gamete is associated with a

[2] Reference is made by number (italic) to "Literature cited," p. 73.

chemical change that can be demonstrated by suitable treatment with iodine (*9*).

It follows that if the chemical nature of the contents of the gametes is influenced by the genes borne by them, an opportunity is presented for external factors to operate as selective agents. This would seem to be the case with the male gametes of maize, and evidence of the operation of selective factors is submitted in the following paper.

It has long been known that the percentage of waxy seeds is often below the expected in crosses where the male parents are heterozygous for this character. The conclusion was reached early that the male gametes bearing the waxy gene were at a disadvantage in effecting fertilization when in competition with gametes bearing the dominant allelomorph. Recently Brink has reported several experiments that confirm the conclusion as to the lower viability of male gametes bearing *wx* (*3*). His experiments show that the smaller proportion of functioning gametes bearing the waxy gene is due to a lower vitality of these gametes. This lower vitality is manifested by a slower rate of pollen-tube growth, when compared with that of the gametes bearing the dominant allelomorph.[3]

Working with comparable material but with a somewhat different viewpoint, the writer has amassed data that fail to support all of Brink's conclusions and that provide still further insight into the behavior of the gametes bearing the *wx* gene. These data have been brought together in the present report. Much of the material is of a disconnected nature, some being fragmentary, but in view of the continued interest in the waxy-nonwaxy segregation it was thought desirable to make all the data available.

Accordingly, the records accumulated over a period of several years have been scanned for their bearing upon the questions raised by others as well as upon those arising in these studies. That the results presented in the following pages occasionally fail to accord with those published by others simply indicates that knowledge of this pair of endosperm factors is as yet imperfect. Doubtless better agreement will follow further investigations, and it is to be hoped that the views here published will meet with sufficient incredulity to stimulate critical experimentation. The study of the waxy character has attained a respectable age, and without apparent loss of vigor, has retained a lively interest.

In the discussion of the behavior of the waxy gene it became distressingly obvious that the very awkward phrase "ratio of waxy to nonwaxy bearing gametes" recurred with disconcerting frequency. To avoid this undesirable expression, the term "nonwaxy" has been omitted wherever possible, and the phrase has been further shortened

[3] Since this paper was prepared, four articles have appeared bearing directly on the subject of gametic selection. Working with shape and color of the seeds in peas, Bond (*2*) shows that when immature stigmas are fertilized with mature pollen from the same plants the proportion of wrinkled to smooth seeds is in excess of the expected, whereas when flowers on the same plants are self-pollinated, wrinkled seeds are below the expected. This difference is attributed to the fact that pollen applied to immature stigmas is in effect stored, since germination is delayed until the stigmas are receptive.

Kiesselbach and Petersen (*8*) have added further data confirming the deficiency of waxy seeds. They conclude that the deficiency is due to a differential pollen tube growth, dependent upon the waxy gene or a gene closely linked with it.

Mangelsdorf and Jones (*10*), in a discussion of the gametophytic factors in plants, suggests that the deficiency of waxy seeds is due to the action of a gametophytic factor located on the chromosome with the waxy gene.

Muller and Settles (*11*) show that, at least for Drosophila, the functioning of the gametes is not affected by the genes they bear. These authors discuss the differences between plants and animals with respect to the sporophytic generation and point out that in the former, environic differences might be expected to result in gametic selection.

by characterizing the gametes by the nature of the genes borne by them, disregarding the fact that the gametes serve only as vehicles for the genes. It is believed that no serious ambiguity results from this abbreviated form of statement, and the reader is spared the repetition of an annoying phrase.

Before presenting the results obtained in deliberate attempts to modify the gametic ratios with respect to the *c* and *wx* genes an analysis will be presented of data, amassed for other purposes, which may be utilized to confirm or refute previous conclusions.

RATIO OF WAXY TO HORNY NOT INVARIABLY LOWER IN THE MALE GAMETES

In a previous paper (*7*) it has been shown that the proportion of waxy to horny seeds is less than the expected when the male parent is heterozygous for the waxy gene and, further, that when the female parent is heterozygous no such deficiency occurs. This conclusion was based on data, only a small part of which involved identical plant reciprocals, and was open to the criticism that the plants furnishing the gametes of one sex were not identical with those furnishing gametes of the other sex.

Recently, with more extensive material, Brink (*3*) has confirmed the conclusion with respect to the deficiency of waxy seeds when the male parent is heterozygous, but his data suffer from the defect mentioned above in that the comparison was not restricted to direct plant reciprocals.

In a study of the factors affecting the rate of crossing over there has been accumulated a series of 151 direct plant reciprocals from 13 closely related progenies. These reciprocal crosses provide material for a further test of the ratio of waxy to horny gametes in the sexes.

None of these ears involved, in a heterozygous condition, either the *su* endosperm or *I* aleurone factors that have been shown by Brink to affect the proportion of waxy gametes.

TABLE 1.—*Comparison of the percentages of waxy gametes in the two sexes; crosses limited to plant reciprocals*

Progeny designation	Number crosses	Female gametes		Male gametes		Difference, female—male
		Number of seeds	Per cent waxy	Number of seeds	Per cent waxy	
Dh 416 L3 L1 C1 L1 L22	4	1,919	49.4±*1.21	1,885	51.1±1.00	−1.7±1.57
Dh 416 L3 L1 C1 L1 L1 L23	7	3,400	49.6± .41	2,104	49.8± .49	−.2± .64
Dh 416 L3 L1 C1 L1 L2 L23	8	3,064	51.2± .68	2,722	49.6± .77	1.6±1.03
Dh 416 L3 L1 C1 L1 L3 L23	7	2,933	48.5± .48	1,387	49.0± .72	−.5± .87
Dh 416 L3 L1 C3 L21	5	2,331	50.1± .46	4,100	56.0±1.55	−5.9±1.62
Dh 416 L3 L1 C3 L1 L22	5	3,296	50.0± .35	2,266	54.1± .63	−4.1± .72
Dh 416 L3 L1 C5 L21	5	4,068	48.7± .67	7,352	48.5± .53	.2± .85
Dh 416 L3 L1 C5 L4 L23	7	3,523	49.8±1.30	3,661	47.7± .77	2.1±1.51
Dh 416 L3 L1 C5 L4 L1 L24	17	7,336	49.2± .36	6,300	46.7± .59	2.5± .69
Dh 416 L3 L1 C5 L3 L1 R23	49	24,627	50.3± .23	27,517	48.1± .31	2.2± .39
Dh 416 L3 L1 C5 L3 L2 R23	11	5,284	49.4± .36	3,683	46.3± .79	3.1± .87
Dh 417 L1 L2 L1 L21	13	4,995	49.9± .56	4,693	50.2± .57	−.3± .80
Dh 417 L1 L2 L1 L2 L23	13	4,122	51.3± .70	3,757	49.3± .61	2.0± .93

* All errors corrected for size of population.

The summarized data for the 13 progenies are presented in Table 1. Inspection shows that there is no consistent deficiency of functioning

waxy gametes in the male sex, and in fact the greatest differences between the sexes in this respect are those where a deficiency of waxy gametes is indicated in the female sex.

From inspection there would seem to be no objection to combining the results from the 13 progenies, but Fisher's (*6*) test for homogeneity clearly shows this array of differences to be heterogeneous.

It would appear then that the earlier conclusion as to the deficiency of waxy seeds when heterozygous plants are used as male parents is applicable only to certain progenies, and the opposite condition may obtain with other material.

However, despite the failure of this material to substantiate earlier conclusions, it is clear that the proportion of functioning waxy gametes in the male sex is more variable than the proportion in the female sex.

If the mean standard deviation of the percentage of waxy seeds is calculated for the two sexes in the reciprocal crosses from the 13 progenies it is found that the $\sigma ♀ = 2.365 \pm 0.150$ and $\sigma ♂ = 2.98 \pm 0.118$, or a difference of 0.615 ± 0.19. Thus the variability of the waxy-horny ratio in the male gametes is 26 per cent greater than that found in female gametes, but this difference only slightly exceeds three times the error.

If the data are treated as paired observations of the standard deviation of male and female gametes, and the mean difference and probable error calculated from the array of differences between the members of each pair, weighted by the reciprocal of the squared probable error of the differences (to correct for populations of varying sizes), the mean difference in standard deviation is found to be 0.572 ± 0.173, a difference of 3.3 times the probable error (when the standard deviation of the array has been corrected for the small size of the population). This latter method of treating the paired observations corresponds very closely to Student's method. The latter shows that a difference of the observed magnitude would not be expected to occur as the result of chance more often than once in 42.5 trials. This is very close to the odds of 37.5 to 1 deduced from the departure of 3.3 times the error.

Further, Fisher's test (*6*) for homogeneity shows that the array of differences is homogeneous.

If there is any intimate connection between the proportion of functioning waxy gametes and the variability of the percentage of such gametes, it should be found that the percentage of waxy gametes is more variable in the male sex when there is a deficiency of such gametes in this sex and, conversely, the variability should be less in the males when the waxy gametes are in excess in this sex. No such agreement is found, and in fact the most variable progeny with respect to the waxy ratio in the male gametes is one in which this ratio was highest in the male gametes.

It has been shown in a previous publication (*5*) that the ratio of waxy to nonwaxy gametes in the male sex is profoundly modified by storage of the pollen for even a few hours, and it seems not unreasonable to infer that shorter periods of storage would have a correspondingly less pronounced effect.

In these experiments the tassels of the plants to be used as male parents were bagged usually one or more days before making the crosses. There is evidence that a small quantity of pollen may remain viable

for 24 hours, and since old pollen has a higher proportion of waxy gametes, there would be a tendency to increase the percentage of waxy seeds over the expected equality, when the parents had been bagged even one day in advance of use. An effect of this nature might well account for the observed gametic ratios in the sexes. It appears that the percentage of waxy gametes is more variable in the male than in the female, but there is no evidence that this variability is associated with a differential viability.

PERCENTAGE OF WAXY SEEDS ALIKE ON UPPER AND LOWER EARS WHEN MALE PARENT IS HETEROZYGOUS

Since in some instances the percentage of waxy seeds is higher when the male parent is heterozygous and in others when the female parent is heterozygous, it seemed desirable to examine other groupings of data not based on sex differences.

TABLE 2.—*Percentage of waxy in upper and lower ears where male parent is heterozygous (Dh 416 L3 L1 C5 L4 L1 L24)*

Plant numbers	Upper ears		Lower ears		Difference
	Total seeds	Per cent waxy	Total seeds	Per cent waxy	Upper—lower
5702×5905	379	46.4±1.73	82	52.4±3.72	−6.0±4.11
5710×5909	170	44.7±2.57	340	50.0±1.83	−5.3±3.16
5725×5931	660	49.9±1.31	593	49.8±1.39	.1±1.91
5759×5912	598	45.7±1.37	583	47.4±1.40	−1.7±1.96
5718×5903	516	50.8±1.48	566	48.1±1.42	2.7±2.05
5743×5950–a	564	54.1±1.42	703	50.1±1.27	4.0±1.91
5781×5972	525	54.7±1.47	568	55.1±1.41	−.4±2.08
5795×5991	266	53.4±2.06	489	54.0±1.52	−.6±2.56
3601×3997	518	46.7±1.48	181	55.2±2.50	−8.5±2.91
3620×3998	364	43.1±1.75	628	43.0±1.33	.1±2.19
3631×3998	596	48.8±1.38	592	49.0±1.39	−.2±1.96
3664×4022	491	42.2±1.50	344	51.4±1.82	−9.2±2.36
Mean					−1.03±.78

TABLE 3.—*Percentage of waxy in upper and lower ears where male parent is heterozygous (Ph 230 L1 R24)*

Plant numbers	Upper ears		Lower ears		Difference
	Total seeds	Per cent waxy	Total seeds	Per cent waxy	Upper—lower
510×616	547	41.7±1.42	282	42.9±1.99	−1.2±2.45
519×654	258	41.5±2.07	226	49.6±1.57	−8.1±2.61
533×723	46	52.2±4.97	402	47.0±1.68	5.2±5.25
540×720	298	50.0±1.96	324	44.1±1.86	5.9±2.71
546×707	423	42.8±1.63	260	41.9±2.07	.9±2.63
548×603	394	40.6±1.67	407	40.3±1.64	.3±2.34
1×101	486	41.8±1.51	509	40.1±1.46	1.7±2.11
3×110	462	51.4±1.06	319	46.3±1.41	5.1±1.76
5×200	128	46.1±2.98	317	51.4±1.90	−5.3±3.53
6×103	708	47.7±1.27	190	36.3±2.36	11.4±2.68
7×136	429	49.0±1.62	528	41.8±1.45	7.2±2.18
12×162	274	46.0±2.06	229	45.0±2.22	1.0±3.01
13×149	8	37.5±10.55	22	36.4±6.92	1.1±13.48
15×126	193	45.1±2.42	36	47.2±5.62	−2.1±6.08
23×169	265	49.4±2.07	274	46.4±2.03	3.0±2.90
27×181	534	49.4±1.46	415	42.2±1.63	7.2±2.19
28×182	465	46.2±1.56	511	52.6±1.49	−6.4±2.16
Mean					2.03±.85

The records afford two progenies in which upper and lower ears were pollinated at the same time with identical lots of heterozygous pollen. The ears are shown in Tables 2 and 3. In neither case do the ears of one position have a percentage of waxy significantly different from those in another position, and in one progeny the difference is in the direction of the upper ears having the highest percentage, while in the other the lower ears have the highest percentage.

PERCENTAGE OF WAXY SEEDS HIGHER ON UPPER THAN ON LOWER EARS WHERE FEMALE PARENT IS HETEROZYGOUS

A third progeny provides data that may be used to detect a difference between upper and lower ears when the female parent was heterozygous and the male parent was homozygous for *wx*.

The ears of this nature are shown in Table 4. The ears were pollinated at the same time, each pair with identical pollen, and the only difference between the members of a pair is that due to their position. The difference in percentage waxy is $2.11 \pm .45$, with the upper ears having the highest percentage. This difference is fully as large as any found in groupings based on sex.

TABLE 4.—*Percentage of waxy in upper and lower ears where female parent is heterozygous (progeny Dh 416 L3 L1 C5 L4 L1—4—L24)*

Plant number	Upper ears			Lower ears			Difference
Heterozygous parent	Total seeds	Number waxy	Per cent waxy	Total seeds	Number waxy	Per cent waxy	Upper–lower
3984	560	275	49.1±1.42	154	71	46.1±2.70	3.0±3.05
3985	698	360	51.6±1.27	448	210	46.9±1.59	4.7±2.04
3986	813	403	49.6±1.18	441	214	48.2±1.60	1.4±1.99
3988	495	238	48.1±1.51	61	30	49.2±4.31	−1.1±5.35
3992	166	91	54.8±2.61	86	50	58.1±3.57	−3.3±4.42
3993	245	119	48.6±2.15	276	120	43.5±2.01	5.1±2.94
3994	355	190	53.5±1.79	444	235	52.9±1.60	.6±2.40
3996	434	217	50.0±1.62	27	14	51.8±6.50	−1.8±6.70
3998	349	176	50.4±1.75	423	215	50.8±1.54	−.4±2.33
3990	242	127	52.5±2.16	554	284	51.3±1.43	1.2±2.59
4000	188	86	43.7±2.45	15	7	46.7±8.70	−1.0±9.02
4004	118	55	46.6±2.89	249	131	52.6±2.13	−6.0±3.59
4008	555	276	49.7±1.43	454	218	48.0±1.58	1.7±2.13
4009	397	199	50.1±1.69	355	164	46.2±1.78	3.9±2.46
4010	136	67	49.3±2.89	410	198	48.3±1.66	1.0±3.33
4020	410	211	47.8±1.66	119	59	52.1±3.08	−4.3±3.50
4022	502	260	51.6±1.50	306	150	44.1±1.91	7.5±2.43
Mean			50.0± .31			48.9± .52	2.11± .45

Upper ears are usually somewhat more advanced than the lower ears, and when both are pollinated at the same time the gametes of the upper ears are at least some hours older than those of the lower ears. If the female gametes are affected by age in the same manner as are the male gametes then the upper ears would be expected to have a higher percentage of waxy than the lower ears.

Although the upper ears in this material have a higher percentage of waxy seeds, this does not conflict with Brink's (*3*) evidence that long styles tend to impair the functioning of waxy-bearing gametes. It is true that ears with long styles are necessarily older than those with short styles, and for this reason would be expected to have a higher percentage of waxy if the results shown in Table 4 are of

general application. However, in Brink's experiments the short-styled controls were obtained by cutting back silks which presumably were of the same length as those used for the long-style parents, and hence the gametes were of the same age. However, it should be noted that in both the long and short styled material the percentage of waxy was below the expected, whereas both sets of ears must have been older at the time of fertilization than normal material, and hence would be expected to have an excess of waxy seeds. A possible explanation of the failure to obtain a higher percentage of waxy on these ears lies in the fact that they were self-pollinated. If the ratio of waxy to nonwaxy in the effective male gametes were less than equality, a condition often met with, this inequality may have more than overbalanced any inequality in the opposite direction resident in the female gametes.

There is no occasion, however, to overstrain the comparison of Brink's experiment with that presented in Table 4, since many more data of a substantiating nature would be necessary to establish the generality of the phenomenon of an increase in effective waxy female gametes with advancing age.

PERCENTAGE OF WAXY SEEDS ALIKE ON BUTTS AND TIPS OF THE EAR

If the pollen tubes of gametes bearing the waxy gene are at a disadvantage in effecting fertilization as compared with those bearing the nonwaxy gene, then it should be found: (1) That the percentage of waxy seeds on the lower halves of the ears is less than that on the upper halves when heterozygous pollen is applied to double recessive silks, but no such difference should be found when double recessive pollen is applied to the silks of heterozygous plants; (2) a correlation should be found between the total number of seeds on an ear and the percentage of waxy seeds when heterozygous pollen is used on double recessive plants, but no correlation is expected where the procedure is reversed, i. e., with homozygous waxy-bearing pollen applied to heterozygous plants.

TABLE 5.—*Percentage of waxy seeds in tip and base of ears where male parent is heterozygous (progeny Dh 416 L3 L1 C5 L3 L1 R23)*

Plant number	Tip		Base		Difference
	Total seeds	Per cent waxy	Total seeds	Per cent waxy	Tip−base
6005	205	53.7±2.35	106	56.6±3.37	−2.9±4.11
6012	140	42.9±2.85	201	44.3±2.37	−1.4±3.71
6014	202	50.0±2.37	180	44.4±2.51	5.6±3.45
6017	160	50.0±2.67	140	50.7±2.85	−.7±3.91
6024	272	39.7±2.05	220	38.6±2.28	1.1±3.07
6016	170	47.1±2.59	123	42.3±3.05	4.8±4.00
6010	225	38.2±2.23	213	43.7±2.31	−5.5±3.21
6025	120	37.5±3.08	149	45.6±2.76	−8.1±4.14
Mean					−0.72± .29

Eight ears are available to test the hypothesis in (1), where heterozygous pollen was applied to double recessive plants (Table 5).

These 8 ears comprise 2,826 seeds. They were divided into upper and lower halves and the two lots classified separately. The ears were not long, but the pollen tubes functioning on the lower halves must have grown on an average about 3 inches further than those fertilizing the ovules of the upper halves of the ears.

The differences between the eight pairs of tips and butts were treated as an array from which the mean and standard deviation (corrected) were calculated; both being weighted by $1/E_d^2$ to correct for differences in number of seeds. Treated in this manner, the mean difference in percentage of waxy seeds between tips and butts is found to be 0.724 ± 0.29 per cent; the butts having on an average 0.7 per cent more waxy seeds than the tips. The difference is not significant, and with the error involved a difference smaller than 1 per cent could not have been established. Since the observed percentage of waxy seeds in Brink's (*3*) long-styled material was 20.44, it follows that 40.8 per cent of the functioning male gametes bore the *wx* gene, whereas in the control short-style material the observed percentage of waxy seeds was 23.95, showing that 47.9 per cent of the functioning male gametes bore the *wx* gene. The difference may be taken to represent the differential effect of the long styles; being in this case 7.1 per cent, or approximately 2 per cent for each increase of 3 inches in length of style above the control series.

Forty-two ears are available to test the second hypothesis of (1) above, where homozygous recessive *wx* pollen is applied to heterozygous plants. These ears fell into four classes with respect to color, and the data have been summarized in Table 6. The mean difference for each color class was determined separately by the method outlined above. Treating the array of differences of the paired values in the several color classes in the same manner as above, the mean difference is found to be 0.132 ± 0.55, with the upper halves of the ear having the highest percentage of waxy seeds. There is little indication, therefore, that the percentage of waxy seeds is higher in the tips than in the butts of ears.

TABLE 6.—*Difference in percentage of waxy seeds in tip and base of ears where female parent is heterozygous (progeny Dh 416 L3 L1 C5 L3 L1–4–R23)*

Nature of cross	Number of ears	Tip-base, mean difference in per cent waxy
Back crossed, 50.0 per cent white	23	−1.33±0.73
Back crossed, 62.5 per cent white	9	1.81±1.32
Self, 25.0 per cent white	3	1.44±1.62
Self, 43.75 per cent white	7	2.92± .84
Mean		.132± .55

The correlation testing (*3*) above has been published (*5*). Considering male gametes alone, a coefficient of 0.161 ± 0.47 is found between the percentages of waxy and the total number of seeds formed when heterozygous waxy pollen is applied, whereas a coefficient of -0.017 ± 0.73 is found between these two variables when the female parent is heterozygous for waxy.

Restricting the population to identical plant reciprocals, and thus insuring the consideration of the same plants in measuring the relationship in the two sexes, the following coefficients are found:

	Per cent waxy in male	Per cent waxy in female
Number of seeds on male	0. 017±0. 98	0. 090±0. 97
Number of seeds on female	. 114± . 86	. 020± . 98

None of these coefficients is significant. It is of interest to observe that in this series of 47 reciprocal crosses between sibs, the correlation between the percentage of waxy seeds when the heterozygous parent is used as a female and that found when these same plants serve as male parents is -0.020 ± 0.98, thus showing that the variations in the percentage of waxy are not due to genetic diversity.

LONG STYLES NOT ASSOCIATED WITH A LOW PERCENTAGE OF WAXY SEEDS

Brink (*3*) has shown that in two lots of self-pollinated ears differing in style length the percentage of waxy seeds is lower in the group having the longest styles.

In the course of an investigation into the factors affecting crossover percentages, paired back-cross pollinations were made on ears of long and short styles and on ears having styles of the same length. In these cases a single plant in each instance furnished the pollen for a given pair of ears.

The data accumulated for the crossover study will serve also to determine the general applicability of Brink's conclusion.

TABLE 7.—*Comparison of the percentage of waxy seeds on ears differing in length of styles and on ears where the styles were of the same length; these ears were borne on homozygous recessive plants*

EARS DIFFERING IN SILK LENGTH					
Long-styled ears		Short-styled ears		Difference	
Length of silk (inches)	Per cent waxy	Length of silk (inches)	Per cent waxy	Length of silk (inches)	Per cent waxy; long-short
3	51. 4±2. 81	¾	46. 1±4. 41	2¼	5. 3±5. 22
4	49. 4±3. 07	2	46. 4±3. 01	2	3. 0±4. 30
3	41. 8±2. 30	2	51. 4±1. 88	1	−9. 6±2. 97
3	46. 2±2. 31	1	52. 6±2. 21	2	−6. 4±3. 19
3	41. 5±3. 07	1	49. 6±3. 33	2	−8. 1±4. 53
7	42. 8±2. 41	4	41. 9±3. 06	3	. 9±3. 90
7	40. 6±2. 47	2	40. 3±2. 43	5	. 3±3. 46
3	46. 0±3. 01	2	45. 0±3. 29	1	1. 0±4. 46
Mean				2. 16	−2. 95±1. 36
EARS OF SAME SILK LENGTH					
1	49. 0±2. 41	1	41. 8±2. 15	0	7. 2±3. 23
1	47. 7±1. 88	1	36. 3±3. 49	0	11. 4±3. 97
1	41. 7±2. 11	1	42. 9±2. 95	0	−1. 2±3. 62
1	50. 0±2. 90	1	44. 1±2. 76	0	5. 9±4. 01

Considering the cases where the male parent was heterozygous for *wx* and the female was homozygous recessive for this factor, the results shown in Table 7 were found. It is apparent that the percentage of waxy seeds on the long-styled ears was lower than that on the short-styled ears, but the same sort of differences are observed where the styles of the pair were of equal length. When large differences within the pair are found they do not seem to be correlated with large differences in silk length.

The difference between the percentage of waxy in the long and short-styled series is not significant, but in amount accords well with that observed by Brink. The failure to find a better agreement between the difference in length of style and that in percentage of waxy raises the question as to whether in this comparison length of style had any effect on the functioning of the gametes having the *wx* gene.

A larger series involving style length where the female parent was heterozygous and the male homozygous *wx* are shown in Table 8.

TABLE 8.—*Comparison of the percentage of waxy seeds on ears differing in length of styles and on ears where the styles were of the same length; these ears were borne on heterozygous plants*

EARS DIFFERING IN SILK LENGTH					
Long-styled ears		Short-styled ears		Difference	
Length of silk (inches)	Length of waxy	Length of silk (inches)	Per cent waxy	Length of silk (inches)	Per cent waxy; long-short
5	46.4±1.91	4	47.4±4.30	1	−1.0±4.70
1	45.4±2.85	½	48.1±2.59	½	−2.7±3.85
3	45.7±2.50	1	50.7±6.11	2	½5.0±6.60
4	45.2±2.74	¾	50.2±2.51	3¼	−5.0±3.72
8	49.5±2.05	¾	47.6±2.13	7¼	1.9±2.95
3	51.0±2.34	1	53.4±4.10	2	−2.4±4.72
8	49.5±1.93	4	48.6±2.73	4	.9±3.34
3	50.3±3.94	1	5308±2.95	2	½3.5±4.92
3	52.2±2.02	1	47.1±2.60	2	5.1±3.29
2	50.0±3.20	1	53.6±2.54	1	−3.6±4.08
4	52.2±2.22	1½	46.4±3.06	2½	5.8±3.77
4	53.4±2.79	1	51.7±4.60	3	1.7±5.38
5	47.6±2.60	3	47.5±4.52	2	.1±5.21
5	50.5±2.28	4	45.5±2.65	1	5.0±3.50
4	50.7±2.60	½	48.5±5.02	3½	2.2±5.64
4	45.6±2.18	1	45.7±2.55	3	−.1±3.35
6	51.5±2.16	4	48.4±2.31	2	3.1±3.16
5	49.3±2.55	1	49.4±2.38	4	−.1±3.43
3	52.0±2.79	1	50.2±3.35	2	1.8±4.35
1	48.9±2.61	½	48.5±2.71	½	.4±3.76
Mean				2.30	.76± .49
EARS OF SAME SILK LENGTH					
2	51.4±2.57	2	54.7±4.40	0	−3.3±5.00
2	53.3±3.88	2	56.4±3.01	0	−3.1±5.01
½	50.7±6.02	½	50.5±5.52	0	−8.8±8.16
1	48.6±2.35	1	52.8±4.46	0	−4.2±5.04
2	36.7±6.22	2	44.4±3.70	0	−7.7±7.22
1	49.7±2.70	1	51.8±2.64	0	−2.1±3.78
1	49.2±2.36	1	48.4±2.46	0	.8±3.41
1	46.7±2.78	1	44.4±4.46	0	2.3±5.26
2	49.4±4.00	2	49.3±1.82	0	.1±4.30
1	51.6±2.58	1	55.3±5.70	0	−3.7±3.15
3	50.8±3.27	3	51.3±5.73	0	−.5±3.73

Here again no significant difference is found either when the pairs differ in style length or when they have styles of the same length.

Variations in the percentage of waxy seeds are found, but these do not seem to be associated with differences in style length.

From the standpoint of differential pollen-tube growth no effect of style length is to be expected in the series of ears shown in Table 8, but it will be observed that differences in the percentage of waxy are found that are entirely comparable with those observed in the series shown in Table 7, where heterozygous pollen was used.

In none of these cases, however, were the differences in style length as great as those of Brink, and the failure to corroborate his conclusions may well be due to this fact. Nevertheless, it should be observed that there are many ears where the waxy class is deficient in numbers, though no great length of style is involved.

GAMETES BEARING THE GENES SU AND WX NO LESS EFFECTIVE THAN THOSE WITH OTHER COMBINATIONS OF THESE GENES

Brink (*3*) found that when plants homozygous for *su* and heterozygous for *Wx* were self-pollinated only 18.84 per cent of the resulting seeds were waxy, whereas the expected percentage was 25. Recognizing that some factor other than *su* may have been involved in his results, he states, "there is considerable justification for the conclusion that the gene for sugary greatly increases the disparity between the observed proportion of nonwaxy and waxy seeds and that expected on a chance basis."

To obtain a deficiency of 6.26 per cent on self-pollinated ears, if the assumption be made that both sexes are affected equally, the gametic proportions must be 56.6 nonwaxy to 43.4 waxy. But if it be assumed that the distorted ratios result from some sort of differential viability operative only in the male gametes, then 25 per cent of the waxy-bearing gametes fail to function, and the effective ratio of nonwaxy to waxy gametes is 62.5 to 37.5.

Since Brink has shown that gametes bearing *wx* are less effective than those bearing *Wx* when the styles are long, it is entirely possible that the pollen tubes of *su wx* gametes have a slower rate of growth than those constituted *Su wx*. If this were a general phenomenon the ratios obtained in all crosses involving *Su* and *Wx* should be unorthodox.

As a first check on the hypothesis of a differential viability of the genes bearing *su wx*, the published data may be examined. Collins and Kempton (*4*) published the seed classes of 51 ears that were the result of self-pollinating plants heterozygous for *Su* and *Wx*. At the time these data were published no method was known for determining the *su wx* group other than breeding tests, and these accordingly were combined with the *su Wx* group, making the expected on a normal hypothesis 9 horny, 3 waxy, 4 sweet. If it be assumed that one-fourth of the *su wx* gametes fail to function, as in Brink's case, the expectation for these ears becomes 9.42 horny, 2.915 waxy, 3.665 sweet.

The observed distribution was:

	Horny	Waxy	Sweet
Observed	12, 728	3, 941	5, 463
Expected 9–3–4	12, 449	4, 150	5, 533
	+279	−209	−70

$X^2 = 17.66$

The departure from the 9–3–4 ratio is too large to be attributed to chance, but since there is a priori knowledge from many other progenies of a deficiency of waxy seeds and, further, since the observed departures are in the classes horny and waxy, the failure to obtain a better fit is clearly due to the defect of the waxy.

Knowing that waxy seeds are generally deficient, and having an observed deficiency in this class of 209 seeds, it is apparent that if the same percentage of defect held for the waxy gene when in combination with *su* the expected deficiency in the *su wx* class would be 69.75 seeds. In the case above the *su wx* class was combined with the *su Wx* class, and this combination was in defect 70 seeds. It is clear that if this whole amount is assumed to be due to a defect of the *su wx* class it is no larger than would have been expected from the behavior of the *Su wx* group, and therefore offers no evidence for a lower viability of gametes the *su wx* combination of genes as compared with those bearing *Su wx*.

Comparing the observed population with that expected on the assumption of a 25 per cent mortality of the *su wx* gametes the following is obtained:

	Horny	Waxy	Sweet
Observed	12, 728	3, 941	5, 463
Expected	13, 026	4, 034	5, 072
	−298	−93	+391

$X^2 = 46.19$

Clearly this fit is much worse than that obtained in the 9–3–4 comparison.

More recent material may be used to examine this matter further. These data are confined to two sister F_2 progenies (Ph 230 L1 and Ph 230 L2).

As the result of crosses of the nature *Su su wx wx* ♀ × *Su su Wx wx* ♂, 29 ears having 7,974 seeds were obtained. These were distributed:

	Horny	Waxy	Sweet	Waxy sweet
Observed	3, 220. 00	2, 632. 00	1, 170. 00	952. 00
Expected 3–3–1–1	2, 990. 25	2, 990. 25	996. 75	996. 75
	+229. 75	−358. 25	+173. 25	−44. 75

$X^2 = 84.86$

A very poor fit, indeed, but one again due largely to the defect of the waxy seeds. Any deficiency in effective *su wx* gametes should be indicated by a lower percentage of waxy seeds in the sweet group when compared with that in the nonsweet group. The percentage is 44.86 in the former and 44.98 in the latter.

The disturbance in these ratios seems to be confined largely to the male gametes, since where the cross is made *Su su Wx wx* ♀ × *Su su wx wx* ♂ the fit to a 3–3–1–1 ratio is as good as could be expected, as is seen in the following comparison:

	Horny	Waxy	Sweet	Waxy sweet
Observed	5, 881. 00	5, 753. 00	2, 023. 00	1, 944. 00
Expected	5, 850. 37	5, 850. 37	1, 950. 10	1, 950. 10
	+30. 60	−97. 37	+72. 90	−6. 1

$$X^2 = 4.5$$
$$P = 0.25$$

Since in these cases abnormal waxy or waxy sweet behavior is being sought, it may be well to consider these two seed classes irrespective of the sweet class. On the hypothesis that the gametes bearing *su wx* are less able to effect fertilization than those with other combinations of these genes, the percentage of both waxy and waxy sweet seeds should be reduced. Thus in back crosses of the nature *Su su wx wx* ♀ × *Su su Wx wx* ♂ the double recessive class, *su wx* should be less than 12.5 per cent.

The progenies considered above provide 90 ears, in which 12.5 per cent of the seeds are expected to be of the double recessive class, *su wx*. These ears were obtained in the two sister progenies Ph 230 L1 and L2 by crossing plants of the nature *Su su wx wx* and *Su su Wx wx*. The percentage of waxy sweet seeds obtained with heterozygous pollen in progeny Ph 230 L1 is 11.95 ± 0.44 and when heterozygous plants of this progeny are used as female parents the percentage is 12.45 ± 0.19. The heterozygous male gametes of progeny Ph 230 L2 gave 11.93 ± 0.46 per cent of *su wx* seeds, whereas heterozygous female gametes of this progeny gave 12.77 ± 0.35 per cent *su wx*. None of these percentages depart significantly from the 12.5 per cent expected if the *su wx* gametes functioned normally, but there is an indication that the percentage of *su wx* seeds is lower when the male parent is heterozygous than when the female is heterozygous.

To test this point further resort must be had to identical plant reciprocals, and these accordingly have been selected from the 90 ears and grouped in Table 9. Only 10 pairs of reciprocals are available, and these show a mean difference of 1.69 ± 0.32, with the percentage of waxy sweet seeds highest where the female parent is heterozygous.

If the assumption be made that 25 per cent of the gametes bearing the combination *su wx* fail to function, then back-crosses of the nature *Su su wx wx* ♀ x *Su su Wx wx* ♂ should give 45.8 per cent of the seeds waxy. As a test of this hypothesis Tables 10 and 12 have been prepared, the data being assembled from progenies Ph 230 L1 and L2.

Presumbly the effect of the *su* gene on the survival of the gametes bearing the waxy genes can come into operation only in the male gametes. Table 10 presents 19 ears where the male gametes are measured. The mean percentage of waxy seeds on these ears is 45.0 ± 0.60, not a bad fit to the 45.8 per cent expected on the hypothesis that 25 per cent of the waxy sweet gametes fail to function.

TABLE 9.—*Percentage of seeds of the double recessive combination waxy and sweet in plant reciprocals*

Plant numbers	Male gametes		Female gametes		Difference
	Total seed	Per cent *su wx*	Total seeds	Per cent *su wx*	Female–male
1×101	995	10.5±0.66	727	13.5±0.86	3.0±1.08
8×162	728	10.2± .76	708	12.7± .85	2.5±1.14
13×149	22	18.2±5.54	797	13.7± .82	−4.5±5.60
20×192	270	12.6±1.36	711	11.8± .82	−.8±1.59
23×169	539	11.5± .93	1,144	12.2± .65	.7±1.14
506×624	68	8.8±2.32	148	11.6±1.77	2.8±9.23
513×746	394	10.4±1.04	49	12.2±3.16	1.8±3.32
519×654	484	10.5± .94	401	11.5±1.07	1.0±1.42
522×644	187	11.8±1.59	310	14.8±1.36	3.0±2.09
535×744	86	11.6±2.33	194	14.4±1.70	2.8±2.88
Mean					1.69± .32

TABLE 10.—*Percentage of waxy seeds on ears segregating for Su (male gametes of Ph 230 L1 and L2)*

Plant numbers	Total seeds	Number waxy	Per cent waxy	Plant number	Total seeds	Number waxy	Per cent waxy
1×101	995	407	40.9±1.05	513×746	394	181	45.9±1.69
5×200	445	219	49.2±1.60	514×646	574	241	42.0±1.39
8×162	728	324	44.5±1.24	516×611	350	152	43.4±1.78
12×162	503	229	45.5±1.49	519×654	484	219	45.2±1.53
20×192	270	124	45.9±2.04	520×671	187	88	47.0±2.46
23×169	539	256	47.9±1.46	532×693	124	68	54.8±3.02
28×182	976	484	49.6±1.08	535×744	86	33	38.4±3.54
501×624	118	56	47.4±3.10	535×743	270	122	45.2±2.04
503×657	348	122	35.0±1.72				
506×624	68	35	51.5±4.08	Mean			45.0± .59
507×657	65	28	43.1±4.14				

However, as has been seen, there are often significant differences in the sexes in the proportion of waxy gametes, and with the ears shown in Table 10 we may be dealing with some such phenomenon. As a check the ears shown in Table 11 have been assembled. These ears do not involve the *Su* gene in a heterozygous condition, and resulted from crosses of the nature *Su Su wx wx* ♀ x *Su Su Wx wx* ♂. The plants bearing them were sibs of those producing the ears in Table 10. The 19 ears in Table 11 have a mean of 44.6 ± 0.60 per cent waxy, a percentage very close, indeed, to that found for the ears in Table 10, thus disposing of the hypothesis that the low percentage found for the ears in Table 10 was due to the lower survival rate of *su wx* gametes in plants heterozygous for *Su* and *Wx*.

The percentage of waxy seeds obtained on ears where the female parent was heterozygous for *Su* and *Wx*, and the male parent was heterozygous for *Su* but homozygous for *wx*, should not depart from the expected equality; and the ears shown in Table 12 are the result

of crosses of this nature. The percentage of waxy is 49.6 ± 0.19, a very close approximation to the expected 50 per cent.

TABLE 11.—*Percentage of waxy seeds on ears not segregating for Su (male gametes Ph 230 L1–1–R24 and L2)*

Plant numbers	Total seeds	Number waxy	Per cent waxy	Plant numbers	Total seeds	Number waxy	Per cent waxy
3×110	781	358	45.8±1.20	510×616	829	349	42.1±1.16
4×232	570	264	46.3±1.41	517×611	243	137	56.4±2.15
6×103	898	407	45.3±1.12	527×739	295	154	52.2±1.96
7×136	957	431	45.0±1.09	531×721	154	78	50.6±2.71
15×126	229	104	45.4±2.22	531×677	274	128	46.7±2.04
16×157	450	167	37.1±1.55	540×720	692	293	42.3±1.26
17×157	554	272	49.1±1.43	546×707	683	290	42.4±1.27
25×147	172	83	48.2±2.56	548×603	801	324	40.4±1.17
27×181	949	439	46.2±1.09				
33×184	329	120	36.5±1.79	Mean			44.6± .60
504×624	42	17	40.5±5.10				

TABLE 12.—*Percentage of waxy seeds on ears segregating for Su (female gametes Ph 230 L1 and L2*

Plant numbers	Total seeds	Number waxy	Per cent waxy	Plant numbers	Total seeds	Number waxy	Per cent waxy
101×1	727	365	50.2±1.25	251×20	198	107	54.0±2.39
103×1	1,099	544	49.5±1.02	252×806	499	249	49.9±1.51
108×807	649	323	49.8±1.32	624×506	148	82	55.4±2.76
117×1	1,016	482	47.4±1.06	644×522	310	158	51.0±1.91
144×29	884	412	46.6±1.13	645×514	666	335	50.3±1.31
149×13	797	381	47.8±1.19	650×533	256	134	52.3±2.10
158×7	720	345	47.9±1.25	654×519	401	203	50.6±1.68
160×33	908	448	49.3±1.12	671×513	833	403	48.4±1.17
162×8	708	345	48.7±1.27	674×807	374	194	51.9±1.75
163×28	1,005	503	50.0±1.06	676×527	266	138	51.9±2.06
164×13	858	419	48.8±1.15	677×519	576	285	49.5±1.40
169×23	1,144	556	48.6± .99	690×527	102	43	42.2±3.30
187×33	436	241	55.3±1.61	693×527	284	156	54.9±2.00
192×20	711	362	50.9±1.27	697×523	102	51	50.0±3.34
200×8	636	326	51.2±1.33	713×535	238	112	47.0±2.18
200×808	281	149	53.0±2.02	744×535	194	92	47.4±2.42
203×14	1,004	494	49.2±1.06	746×513	49	24	49.0±4.81
216×5	978	492	50.3±1.08	748×535	137	68	49.6±2.88
222×1	683	338	49.5±1.29	749×535	369	184	49.9±1.75
224×1	376	189	50.3±1.74				
234×36	180	90	50.0±2.50	Mean			49.6± .19
239×33	533	258	48.4±1.46				

In Table 13 are listed the ears produced by crosses of the nature *Su Su Wx wx* ♀ × *Su Su wx wx* ♂. The plants producing these ears are sibs of those that bore the ears shown in Tables 10, 11, and 12. No departure from equality in the proportion of waxy to nonwaxy seeds is expected and none was found, the percentage being 48.9 ± 0.37.

It is apparent from these tables that in this progeny the percentage of waxy seeds resulting from the use of heterozygous pollen on plants homozygous recessive for this character is below equality, and less than that obtained where the crosses are reversed, i. e., the male parent homozygous recessive and the female heterozygous for waxy.

To determine this point directly recourse may be had to direct plant reciprocals. These are shown in Tables 14 and 15. Table 14 lists reciprocal crosses where both *Su* and *Wx* are involved, and Table 15 lists only those crosses where *Su* was homozygous dominant. In both instances the proportion of effective waxy gametes is highest

in the female. It should be observed, however, that the difference between the sexes is least where *Su* is heterozygous, a condition contrary to that expected if Brink's observations as to the low viability of gametes bearing *su wx* were general.

TABLE 13.—*Percentage of waxy seeds on ears not segregating for Su (female gametes Ph 230 L1 and L2)*

Plant numbers	Total seeds	Number waxy	Per cent waxy	Plant numbers	Total seeds	Number waxy	Per cent waxy
110×3	470	236	50.2±1.55	236×28	661	336	50.8±1.31
114-a×35	174	82	47.1±2.56	602×504	773	388	50.2±1.19
119×10	1,045	481	46.0±1.07	603×548	117	51	43.6±3.08
120×6	703	357	50.8±1.27	604×514	211	100	47.4±2.32
126×8	816	380	46.6±1.17	606×534	153	77	50.3±2.72
136×7	676	317	46.9±1.29	615×548	85	47	55.3±3.64
139×35	507	265	52.3±1.49	616×510	438	232	53.0±1.61
147×25	463	215	46.4±1.56	629×546	66	30	45.4±4.13
157×16	728	349	47.9±1.25	631×546	139	84	60.4±2.81
178×26	826	408	49.4±1.17	656×519	57	31	54.4±4.45
179×26	544	279	51.3±1.44	657×521	276	147	53.3±2.02
181×27	452	236	52.2±1.58	662×517	209	106	50.7±2.33
184×33	603	241	40.0±1.34	673×519	308	154	50.0±1.92
191×34	445	205	46.1±1.59	683×527	185	90	48.6±2.48
192×26	461	221	47.9±1.57	684×533	240	102	42.5±2.15
199×15	567	270	47.6±1.41	707×546	490	233	47.6±1.52
208×9	374	189	50.5±1.74	716×531	490	244	50.0±1.52
213×3	447	235	52.6±1.59	720×540	386	193	50.0±1.72
219×36	628	328	52.2±1.35	750×530	46	27	58.7±4.92
230×2	352	183	52.0±1.79				
232×4	366	146	39.9±1.72	Mean			48.9±0.37

TABLE 14.—*Percentage of waxy seeds in reciprocal crosses involving heterozygous Su*

Plant numbers	Male gametes			Female gametes			Difference, female–male
	Total seeds	Number waxy	Per cent waxy	Total seeds	Number waxy	Per cent waxy	
1×101	995	407	40.9±1.05	727	365	50.2±1.25	9.3±1.63
8×162	728	324	44.5±1.24	708	346	48.9±1.27	4.4±1.77
13×149	22	8	36.4±1.21	797	381	47.8±1.19	11.4±1.70
20×192	270	124	45.9±2.04	711	362	50.9±1.26	5.0±2.40
23×169	539	258	47.9±1.45	1,144	556	48.6±.99	.7±1.76
506×624	68	35	51.5±4.08	148	82	55.4±2.76	3.9±4.92
513×746	394	181	45.9±1.69	49	24	49.0±4.81	3.1±5.10
519×654	484	219	45.2±1.54	401	203	50.6±1.68	5.4±2.28
522×644	187	88	47.0±2.46	310	158	51.0±1.91	4.0±3.12
535×744	86	33	38.4±3.54	194	92	47.4±2.42	9.0±4.29
Mean							6.18±.84

TABLE 15.—*Percentage of waxy seeds in reciprocal crosses involving homozygous Su*

Plant numbers	Male gametes			Female gametes			Difference, female–male
	Total seeds	Number waxy	Per cent waxy	Total seeds	Number waxy	Per cent waxy	
3×110	781	358	45.8±1.77	470	236	50.2±1.55	4.4±2.35
4×232	570	264	46.3±1.41	366	146	39.9±1.72	−6.4±2.22
7×136	957	431	45.0±1.09	676	317	46.9±1.30	1.9±1.70
16×157	450	167	37.1±1.54	728	349	47.9±1.25	10.8±1.98
35×114	402	197	49.0±1.68	174	82	47.1±2.56	−1.9±3.06
510×616	829	349	42.1±1.16	438	232	53.0±1.61	10.9±1.98
540×720	622	293	47.1±1.35	386	193	50.0±1.71	2.9±2.18
546×707	683	290	42.4±1.27	490	233	47.5±1.52	5.1±1.92
548×603	801	324	40.4±1.17	117	51	43.6±3.09	3.2±3.31
Mean							4.37±1.7

The present material, therefore, offers no support for the hypothesis that male gametes bearing the genes *su* and *wx* are at a disadvantage in effecting fertilization when in competition with gametes bearing other combinations of the genes for sugary and waxy endosperm.

POLLEN STORAGE INCREASES THE PROPORTION OF FUNCTIONING WAXY GAMETES

In an attempt to alter the crossover ratio between the aleurone factor *C* and the waxy endosperm factor *Wx*, pollen from plants heterozygous for these linked genes was stored in the collection bags, in the field, for a period approximating seven hours. As a control, approximately one-half of the pollen collected from each plant was applied immediately to the silks of double recessive plants.

Thirteen successful paired pollinations were obtained, and these showed clearly that although the crossover ratio was unaffected (*5*), the proportion of waxy to nonwaxy gametes was profoundly altered. The functioning waxy-bearing gametes in the stored pollen were in excess of the expected equality and greatly exceeded the proportion of functioning waxy-bearing gametes in the unstored pollen; the latter being less than equality.

Similarly, there was an apparent alteration of the proportion of *C* to *c*-bearing gametes, resulting in an increase in the proportion of white to colored seeds when stored pollen was used. In the cross used, the increase in the proportion of white seeds might be attributed entirely to the linkage between *C* and *Wx*. The parental combinations were *C Wx* and *c wx*, and there was no means of determining whether the observed effect on the ratio of white to colored seeds was the result of linkage with waxy or whether storage actually resulted in a selective action on the *c*-bearing gametes. Accordingly, a somewhat more elaborate experiment was undertaken with a repetition of the original procedure, using in addition a cross in which *C* and *wx* were linked.

All four classes of seed from each cross were planted. The pollen from plants heterozygous for both *C* and *Wx* was used as before in pairs of stored and unstored samples on the double recessive plants. Further, pollen from plants heterozygous for *C* but homozygous for *wx* was applied to plants homozygous for *c* but heterozygous for *Wx*, and the reverse combinations were made also. These latter provide data to measure the effect of storage upon *C* and *Wx* separately.

The same procedure was followed with the cross in which *C* and *wx* were linked.

Considering first the cross in which *C* and *Wx* are linked and where the pollen from the double heterozygote is applied to the double recessive, there are 44 pairs of ears resulting from stored and unstored pollen. The period of storage varied from 6 hours and 10 minutes to 10 hours and 20 minutes, with a mean period of 7 hours and 9 minutes. The ears resulting from stored pollen had on an average 56.8 less seeds than those obtained when the pollen was fresh, which may be taken as a rough approximation of the effect of storage on viability; the period of storage reducing the viability 16.5 per cent.

The data for these 44 pairs are shown in Table 16.

The data presented in Table 16 duplicate, extend, and corroborate those published previously. In measuring the effect of pollen storage

the means and errors are calculated from the arrays of differences between fresh and stored pollen, each difference being weighted by the reciprocal of the squared error of the difference to correct for differences in number of seeds per ear. The procedure followed has been outlined by Collins and Kempton (*5, p. 5–6*).

Before considering the indicated effect of pollen storage, attention should be directed to the fact that Fisher's (*6*) test for homogeneity shows that the arrays of differences between the paired samples are not homogeneous, from which it must be concluded that storage is not in itself the cause of the observed differences in gametic proportions. Such a conclusion is not illogical, since a period of storage simply provides an opportunity for unrelated factors such as temperature, light, and humidity to operate; and the effect of such factors might be measured very imperfectly by time of storage.

With this reservation in mind as to the effect of storage the data may be examined. There is a mean increase of 9.35 ± 1.26 per cent of waxy seeds and an increase of 1.74 ± 0.67 per cent of white seeds when stored pollen is applied. Both the white and waxy seeds are below the expected equality in the series of ears resulting from fresh pollen and in excess in the series obtained with stored pollen.

Since white aleurone and waxy endosperm are known to be correlated in this material, owing to the genetic linkage between the *C* and *Wx* genes, any selective agent operating upon one would be expected to affect the other.

In the 44 ears obtained with fresh pollen there is a correlation of 0.503 ± 0.76 between the percentage of white and the percentage of waxy with a regression of white on waxy of 0.272 and of waxy on white of 0.686.

The regressions can be used to predict the change expected in one ratio from a given change in the other. If this be done it is found that increasing the percentage of waxy seeds by the observed amount of 9.35 would be expected to increase the percentage of white seeds by 2.54. The actual increase observed in the percentage of white seeds was 1.74 ± 0.67, being somewhat, though not significantly, less than the predicted. If the regression of waxy on white be used it is found that having increased the percentage of white seeds by 1.74 an increase of 1.19 in the percentage of waxy would be expected to follow, whereas the increase was 9.35. Clearly the selective action is operative on the gametes bearing the *wx* gene and not on those bearing the aleurone color gene *c*.

As a further measure of the correctness of this conclusion, the regressions of white on waxy and waxy on white may be examined for the array of ears resulting from stored pollen.

If the gametes bearing the waxy gene are operated upon by some selective agent and the departure of the colored-white ratio from equality is due to the linkage between *C* and *Wx*, then the regression of white on waxy should not differ in fresh and stored pollen, but the regression of waxy on white should be much greater in the stored than in the fresh pollen. Both of these conditions are fulfilled. The regression of white on waxy in the stored pollen is found to be 0.276, a very close agreement with the 0.272 found in the fresh pollen, and the regression of waxy on white in the stored pollen is 1.88, whereas in the fresh pollen a regression of but 0.686 was found.

TABLE 16.—*Comparison of ears resulting from fresh and stored pollen (c c wx wx ♀ × C c Wx wx ♂); the linkage in this comparison is between c and wx (progeny Dh 416 L3 L1 C5 L4 L1 L1 L25)*

Date	Fresh pollen							Stored pollen						Differences				
	Time, a. m.	Female No.	Male No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over	Time, p. m.	Female No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over	Per cent white, p. m.-a. m.	Per cent waxy, p. m.-a. m.	Per cent crossing over, p. m.-a. m.	Time,[a] p. m.-a. m.	Seeds, a. m.-p. m.
Aug. 22	9.00	1584–2	1877	267	46.1±2.05	50.6±2.06	35.8±1.97	4.55	1582	395	50.9±1.70	71.1±1.53	37.5±1.64	4.8±2.85	20.5±2.56	1.7±2.57	7.55	−128
Aug. 17	9.10	1590	1861	370	48.9±1.75	50.0±1.75	40.0±1.72	5.00	1592	254	50.8±2.12	50.0±2.15	43.3±2.09	1.9±2.75	0.0±2.77	3.3±2.71	7.50	116
Do	9.10	1595	1862	153	49.7±2.72	37.9±2.64	40.0±2.66	5.00	1602	265	47.9±2.06	53.2±2.06	38.2±2.01	−1.8±4.06	15.3±3.35	−1.8±3.34	7.50	−112
Aug. 20	7.40	1638	1900–D	66	56.1±4.10	50.0±4.15	48.4±4.14	6.00	1597	104	55.8±3.28	66.3±3.12	33.8±3.12	−.3±5.26	16.3±5.19	−14.6±5.18	10.20	−38
Aug. 22	9.10	1663	1878	240	46.7±2.16	18.3±1.68	43.8±2.19	4.50	1602	194	56.2±2.40	84.0±1.77	33.7±2.28	9.5±3.22	65.7±2.43	−10.1±2.89	7.40	46
Aug. 16	9.25	1607–2	1868	419	48.9±1.65	45.1±1.64	38.2±1.60	4.40	1613–2	349	50.7±1.80	57.0±1.78	34.2±1.70	1.8±2.44	11.9±2.42	−4.0±2.33	7.15	70
Aug. 14	10.35	1715	1893	449	62.2±1.54	53.9±1.58	38.5±1.55	5.00	1715	513	48.3±1.49	49.7±1.49	34.5±1.18	−13.9±2.14	−4.2±2.17	−4.0±1.95	6.25	−64
Aug. 19	7.50	1616	1878	388	42.0±1.69	17.3±1.29	45.6±1.76	5.00	1680–2	180	53.9±2.50	53.9±2.50	42.5±2.48	11.9±3.02	36.6±3.15	−3.1±2.80	9.10	208
Aug. 16	9.30	1618–2	1882	148	41.9±2.73	44.6±2.75	37.0±2.66	4.40	1628	85	52.9±3.64	64.7±3.50	45.2±3.64	11.0±4.54	20.1±4.45	8.2±4.50	7.10	63
Aug. 17	9.25	1627	1899	152	44.7±2.70	40.8±2.69	44.1±2.70	5.00	1627–2	60	65.0±4.15	83.3±3.24	46.8±4.34	20.3±4.95	42.5±4.21	2.7±5.10	7.35	92
Aug. 16	9.15	1637	1881	59	54.2±4.35	42.4±4.33	34.3±4.16	4.40	1637–2	246	57.7±2.12	71.1±1.94	43.0±2.12	3.5±4.84	28.7±4.74	8.7±4.66	7.25	−187
Aug. 19	10.15	1640	1862	433	43.4±1.60	40.9±1.59	38.8±1.56	5.00	1640–2	404	45.3±1.67	39.6±1.64	40.4±1.65	1.9±2.32	−1.3±2.28	1.6±2.27	6.45	29
Do	10.15	1641	1880	381	45.4±1.72	46.4±1.72	42.3±1.70	5.00	1644–2	197	49.2±2.40	60.4±2.34	36.8±2.31	3.8±2.95	14.0±2.90	−5.5±2.87	6.45	184
Aug. 17	9.10	1643	1881	368	44.3±1.75	41.0±1.73	36.1±1.69	5.05	1644	63	47.6±4.22	66.7±4.00	31.5±3.94	3.3±4.55	25.7±4.35	−4.6±4.28	7.55	305
Aug. 15	10.00	1645	1861	554	51.8±1.43	44.6±1.42	36.9±1.37	4.30	1646	244	52.4±2.15	47.1±2.15	43.7±2.14	.6±2.57	2.5±2.58	6.8±2.54	6.30	310
Aug. 16	9.15	1648	1862	149	53.0±2.74	40.9±2.71	36.4±2.66	4.40	1645–2	316	60.8±2.30	70.6±1.73	40.3±1.86	7.8±3.57	29.7±3.21	3.9±3.25	7.25	−167
Do	9.15	1651–2	1894	190	48.9±2.44	37.4±2.36	37.3±2.36	4.40	1648–2	94	64.9±3.30	90.4±2.04	44.6±3.46	16.0±4.10	53.0±3.12	7.3±4.19	7.25	96
Aug. 15	10.00	1651	1862	276	53.3±2.01	39.8±1.98	38.2±1.97	4.30	1656	388	50.5±1.70	54.1±1.70	37.1±1.65	−2.8±2.63	14.3±2.61	−1.1±2.57	6.30	−112
Do	10.05	1656–2	1882	664	51.2±1.31	47.6±1.30	39.9±1.28	4.30	1658	389	51.4±1.70	53.0±1.70	33.2±1.61	.2±2.15	5.4±2.14	−6.7±2.06	6.25	275
Aug. 16	9.10	1662	1899	225	48.0±2.24	43.6±2.22	37.4±2.18	4.40	1657	145	49.6±2.80	49.6±2.80	40.0±2.74	1.6±3.58	6.0±2.57	2.6±3.50	7.30	80
Aug. 15	10.05	1658–2	1868	239	42.7±2.16	41.4±2.14	38.9±2.12	4.30	1659	624	50.5±1.35	47.8±1.35	35.4±1.28	7.8±2.54	6.4±2.53	−3.5±2.47	6.25	−385
Do	10.05	1659–2	1886	603	47.6±1.30	45.9±1.35	34.9±1.30	4.30	1660	682	50.6±1.29	52.2±1.29	36.2±1.24	3.0±1.83	6.3±1.86	1.3±1.80	6.25	−79
Do	10.10	1664	1893	417	47.7±1.57	39.6±1.61	30.7±1.52	4.30	1665	67	41.8±4.05	43.3±4.06	28.7±3.70	−5.9±4.34	3.7±4.37	−2.0±4.00	6.20	350
Do	10.10	1666	1894	447	45.2±1.58	46.1±1.59	36.0±1.52	4.30	1666–2	505	52.5±1.49	53.1±1.50	39.5±1.46	7.3±2.17	7.0±2.19	3.5±2.11	6.20	−58
Aug. 14	10.30	1667	1876	417	53.2±1.64	43.6±1.64	35.7±1.57	4.55	1668	79	51.9±3.78	41.8±3.72	34.7±3.60	−1.3±4.12	−1.8±4.07	−1.0±3.93	6.25	338
Aug. 20	7.40	1679	1878	111	55.0±3.18	43.2±3.16	39.5±3.12	6.00	1678	60	51.7±4.34	40.0±4.26	51.3±4.34	−3.3±5.38	−3.2±5.30	11.8±1.69	10.20	51
Aug. 17	9.25	1682	1895	425	45.2±1.62	45.9±1.63	38.6±1.59	5.00	1683	404	50.2±1.68	54.0±1.67	40.3±1.64	5.0±2.33	8.1±2.33	1.7±2.28	7.35	21
Aug. 22	9.10	1684–2	1900	393	43.2±1.68	41.5±1.67	37.1±1.64	4.50	1684	290	46.6±1.96	60.3±1.93	36.4±1.90	3.4±2.58	18.8±2.55	−.7±2.51	7.40	103
Aug. 15	10.15	1686	1897	370	51.4±1.80	50.0±1.80	35.7±1.72	4.35	1692	126	51.6±3.00	57.1±2.97	37.3±2.89	.2±3.50	7.1±3.47	1.6±3.36	6.20	224
Aug. 19	10.25	1693	1899	88	44.3±3.57	43.2±3.54	47.3±3.58	5.05	1697	303	50.5±1.96	45.5±1.93	37.1±1.86	6.2±3.99	2.3±4.04	−10.2±4.03	6.40	−215
Do	10.25	1697–2	1896	458	53.3±1.57	46.3±1.57	36.7±1.54	5.05	1698	464	51.3±1.56	47.0±1.56	39.5±1.56	2.0±2.21	.7±2.21	2.8±2.19	6.40	−6
Aug. 15	10.25	1701	1876	589	50.9±1.39	51.1±1.39	36.8±1.34	4.35	1701–2	173	49.7±2.06	52.0±2.56	30.0±2.34	−1.2±2.91	.9±2.91	−6.8±2.70	6.10	416
Do	10.25	1702	1900–C	738	46.9±1.24	33.9±1.17	46.4±1.24	4.35	1702–2	322	54.0±1.87	60.2±1.83	31.0±1.73	7.1±2.24	26.3±2.17	−15.4±2.13	6.10	416
Do	10.25	1704	1900–B	753	48.9±1.20	50.2±1.23	32.5±1.14	4.35	1704–2	206	48.5±2.34	47.6±2.34	31.0±2.16	−.4±2.62	−2.6±2.64	−1.5±2.44	6.10	547
Do	10.25	1707	1875	345	54.5±1.80	45.8±1.80	40.0±1.78	4.35	1705	614	47.7±1.35	48.2±1.36	39.0±1.32	−6.8±2.25	2.4±2.26	−1.0±2.22	6.10	−269
Aug. 14	10.00	1710–2	1882	37	35.1±5.29	35.1±5.26	53.7±5.50	5.00	1710	592	45.9±1.37	44.8±1.37	39.3±1.35	10.8±5.47	9.7±1.72	−14.4±5.66	6.20	−555
Do	10.35	1711	1881	286	45.4±1.98	34.6±1.90	40.0±2.00	5.00	1711–2	543	47.9±1.44	51.0±1.44	36.9±1.39	2.5±2.45	16.4±2.38	−3.1±2.43	6.25	−257
Do	10.35	1715	1893	449	62.2±1.54	53.9±1.58	38.5±1.55	5.00	1715–2	228	49.6±2.22	46.0±2.24	32.2±2.11	−12.6±2.70	−7.0±2.72	−4.3±2.62	6.25	221
Aug. 16	9.10	1716	1875	322	36.6±1.81	33.8±1.77	52.2±1.87	4.45	1716–2	121	53.7±3.06	65.3±2.92	37.4±2.96	17.1±3.56	31.5±3.42	−14.8±3.50	7.35	201
Aug. 14	10.35	1717	1900–B	521	57.0±1.46	54.3±1.46	46.2±1.47	5.00	1725	375	49.9±1.73	51.5±1.73	36.3±1.67	−7.1±2.27	−2.8±2.27	−9.9±2.22	5.23	146

Aug. 14	10. 35	1717	1900–B	521	57. 0±1. 46	54. 3±1. 46	46. 2±1. 47	5. 00	1725	375	49. 9±1. 73	51. 5±1. 73	36. 3±1. 67	−7. 1±2. 27	−2. 8±2. 27	−9. 9±2. 22	3. 25	146	1
Aug. 15	10. 15	1719	1899	609	47. 3±1. 36	47. 0±1. 36	36. 5±1. 31	4. 35	1718	229	59. 0±2. 19	56. 8±2. 20	36. 0±2. 14	11. 7±2. 58	9. 8±2. 59	−. 5±2. 51	6. 20	380	
Aug. 16	9. 10	1721	1900–C	59	52. 5±4. 38	40. 7±4. 30	27. 0±3. 88	4. 45	1720	145	49. 0±2. 80	68. 3±2. 60	29. 3±2. 54	−3. 5±5. 19	27. 6±5. 02	2. 3±4. 64	7. 35	−86	
Do	9. 10	1723	1878	223	49. 8±2. 24	46. 6±2. 24	42. 6±2. 22	4. 45	1722	305	51. 8±1. 93	49. 8±1. 93	31. 3±1. 78	2. 0±2. 95	3. 2±2. 95	−11. 3±2. 84	7. 35	−82	
Aug. 17	9. 25	1724	1878	366	45. 4±1. 75	47. 3±1. 75	38. 7±1. 71	5. 05	1721–2	360	43. 9±1. 76	45. 3±1. 76	42. 9±1. 75	−1. 5±2. 48	−2. 0±2. 48	4. 2±2. 44	7. 40	6	
Mean				345	49. 0± . 53	44. 1± . 78	38. 8± . 55			289	50. 6± . 38	53. 7± . 94	37. 4± . 38	1. 74± . 67	9. 35±1. 26	−2. 01±5. 52	7. 09	56	

[a] Time is given in hours and minutes.

TABLE 17.—*Comparison of ears resulting from fresh and stored pollen (c c wx wx ♂ × C c Wx wx ♂; the linkage in this comparison is between C and wx (progeny Dh 416 L3 L1 C5 L4 L1 L2 L25)*

Date	Fresh pollen							Stored pollen						Differences				
	Time, a. m.	Female No.	Male No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over	Time, p. m.	Female No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over	Per cent white, p. m.–a. m.	Per cent waxy, p. m.–a. m.	Per cent crossing over, p. m.–a. m.	Time,[a] p. m.–a. m.	Seeds, a. m.–p. m.
Aug. 19	10. 00	1583	2108	225	50. 7±2. 24	43. 6±2. 22	37. 7±2. 18	5. 00	1583–2	295	48. 8±1. 96	51. 9±1. 96	29. 2±1. 78	−1. 9±2. 98	8. 3±2. 96	−8. 5±2. 81	7. 00	−70	
Do	10. 00	1603–2	2118	297	54. 2±1. 94	47. 8±1. 95	32. 8±1. 83	5. 00	1603	309	51. 1±1. 92	42. 7±1. 90	36. 5±1. 85	−3. 1±2. 73	−5. 1±2. 72	3. 7±2. 60	7. 00	−12	
Do	10. 00	1614–2	2090	417	52. 0±1. 65	46. 3±1. 64	31. 4±1. 53	5. 00	1614	261	52. 9±2. 07	44. 1±2. 06	31. 4±1. 93	. 9±2. 65	−2. 2±2. 63	0. 0±2. 46	7. 00	156	
Aug. 15	10. 35	1624–2	2092	422	51. 9±1. 64	45. 0±1. 63	30. 0±1. 50	4. 30	1621	345	43. 8±1. 80	55. 9±1. 63	28. 3±1. 63	−8. 1±2. 43	10. 9±2. 31	−1. 7±2. 22	5. 55	77	
Aug. 14	10. 20	1626	2102	665	49. 6±1. 30	46. 3±1. 30	30. 0±1. 19	4. 50	1676	453	52. 5±1. 58	49. 9±1. 58	31. 8±1. 47	2. 9±2. 05	3. 6±2. 05	1. 8±1. 89	6. 30	212	
Do	10. 20	1642	2093	386	50. 8±1. 71	43. 5±1. 69	31. 3±1. 59	5. 00	1642–2	91	45. 0±3. 50	52. 7±3. 52	35. 3±3. 22	−5. 8±3. 90	9. 2±3. 91	4. 0±3. 59	6. 40	295	
Do	10. 25	1649–2	2092	37	56. 8±5. 50	40. 5±5. 40	24. 5±4. 75	4. 55	1649	536	50. 0±1. 45	44. 0±1. 44	30. 5±1. 34	−6. 8±5. 68	3. 5±5. 59	6. 0±4. 93	6. 30	−499	
Aug. 15	10. 35	1695–2	2117	178	50. 0±2. 52	49. 4±2. 53	29. 7±2. 30	4. 35	1699	519	49. 9±1. 48	53. 4±1. 46	31. 5±1. 37	−. 1±2. 92	4. 0±2. 92	1. 8±2. 68	6. 00	−341	
Aug. 16	9. 45	1903	2110	313	53. 0±1. 90	48. 6±1. 91	28. 3±1. 71	4. 45	1909–2	423	46. 8±1. 63	50. 1±1. 63	30. 0±1. 50	−6. 2±2. 51	1. 5±2. 51	1. 7±2. 27	7. 00	−110	
Aug. 15	10. 30	1906	2103	790	51. 9±1. 19	50. 4±1. 20	32. 3±1. 12	4. 50	1909	518	51. 4±1. 48	48. 3±1. 48	32. 3±1. 39	−. 5±1. 90	−2. 1±1. 91	0. 0±1. 78	6. 20	272	
Aug. 17	9. 55	1906–2	2100	163	52. 1±2. 64	43. 6±2. 62	40. 0±2. 58	5. 10	1912	424	31. 4±1. 51	47. 4±1. 63	52. 7±1. 63	−20. 7±3. 04	3. 8±3. 08	12. 7±3. 05	7. 15	−261	
Do	9. 55	1913	2110	127	59. 0±2. 94	35. 4±2. 84	26. 5±2. 63	5. 10	1914	460	51. 1±1. 57	48. 5±1. 57	28. 0±1. 40	9−7. 9±3. 33	13. 1±3. 24	1. 5±2. 98	7. 15	−333	
Do	10. 00	1914–2	2108	117	52. 1±3. 12	42. 7±3. 08	30. 5±3. 10	5. 10	1915	114	52. 6±3. 14	50. 9±3. 16	33. 2±2. 98	. 5±4. 42	8. 2±4. 41	2. 7±4. 29	7. 10	3	
Aug. 14	11. 10	1917	2113	370	55. 1±1. 74	42. 7±1. 73	32. 1±1. 63	5. 15	1917–2	120	54. 2±3. 06	69. 2±2. 84	57. 9±3. 03	−. 9±3. 52	26. 5±3. 32	25. 8±3. 44	6. 05	250	
Aug. 17	9. 55	1919–2	2117	293	50. 2±1. 97	43. 7±1. 95	33. 0±1. 85	5. 15	1919	229	36. 7±2. 14	68. 6±2. 06	28. 5±2. 00	−13. 5±2. 91	24. 9±2. 83	−4. 5±2. 72	7. 20	64	
Do	9. 50	1926	2094	149	41. 6±2. 72	47. 6±2. 74	28. 0±2. 48	5. 15	1922	182	53. 3±2. 48	55. 5±2. 48	37. 8±2. 42	11. 7±3. 68	7. 9±3. 70	9. 8±3. 47	7. 25	−33	
Aug. 14	11. 15	1923	2088	285	54. 7±1. 98	45. 6±1. 98	34. 3±1. 90	5. 15	1933	542	44. 1±1. 43	53. 9±1. 43	36. 6±1. 39	−10. 6±2. 44	8. 3±2. 44	2. 3±2. 35	6. 00	−257	
Aug. 17	9. 50	1929	2118	364	49. 4±1. 77	42. 8±1. 75	35. 3±1. 68	5. 15	1928	309	44. 7±1. 90	62. 4±1. 86	33. 3±1. 81	−4. 7±2. 59	19. 6±2. 55	−2. 0±2. 47	7. 25	55	
Aug. 19	10. 00	1941	2110	158	53. 8±2. 66	54. 4±2. 67	28. 7±2. 42	5. 20	1928–2	263	41. 1±2. 04	61. 2±2. 02	29. 7±1. 89	−12. 7±3. 35	6. 8±3. 35	1. 0±3. 07	7. 20	−105	
Aug. 22	9. 00	1932	2095	183	50. 3±2. 49	53. 6±2. 48	38. 8±2. 42	5. 05	1937	16	56. 2±8. 36	68. 8±7. 80	31. 3±7. 80	5. 9±8. 71	15. 2±8. 18	−7. 5±8. 16	8. 05	167	
Aug. 15	10. 30	1936	2106	469	52. 4±1. 55	45. 6±1. 55	37. 0±1. 50	4. 50	1936–2	488	54. 7±1. 53	44. 0±1. 51	37. 6±1. 47	2. 3±2. 18	−1. 6±2. 17	. 6±2. 10	6. 20	−19	
Aug. 19	10. 00	1943–2	2091	239	55. 2±2. 16	47. 7±2. 17	34. 8±2. 07	5. 20	1941	147	53. 1±2. 76	50. 3±2. 77	27. 5±2. 48	−2. 1±3. 52	2. 6±3. 52	−7. 3±3. 23	7. 20	92	
Do	10. 00	1951	2094	313	56. 5±1. 89	42. 8±1. 88	36. 3±1. 83	5. 20	1951–2	308	57. 8±1. 89	48. 4±1. 92	35. 3±1. 83	1. 3±2. 67	5. 6±2. 69	−1. 0±2. 59	7. 20	5	
Mean				302	52. 1± . 37	46. 1± . 47	32. 6± . 44			319	48. 3± . 87	51. 1± . 90	33. 8± . 91	−3. 4± . 89	6. 1±1. 07	1. 7± . 80	6. 52	−17	

[a] Time is given in hours and minutes.

If the conclusion be correct that the proportion of gametes bearing the *c* gene to those bearing its dominant allelomorph *C* is altered by pollen storage only as the result of this gene being linked with *Wx*, then it must follow that when the linkage is reversed (*C* being associated with *wx*) an increase in the percentage of waxy seeds must be accompanied by a *decrease* in the percentage of white seeds.

To determine the correctness of this assumption the data shown in Table 17 have been compiled. The ears shown in this table were obtained by the same procedure as was followed where the linked factors were *C Wx*, and the biometrical constants have been calculated as described for the 44 pairs of pollinations shown in Table 16. There is a slight difference between the two sets of data in that the period of storage was less for the ears shown in Table 17 than for those in Table 16. This difference in time was unavoidable since the progeny that produced the 44 pairs of ears was pollinated first and the time that elapsed in handling this progeny necessarily was at the expense of the succeeding progenies. Since the 44 pairs were a direct repetition of the original material the emphasis was placed on obtaining adequate data from this progeny first. The mean time of storage for the ears shown in Table 17 was 6 hours and 52 minutes as compared with a period of 7 hours and 9 minutes for the ears in Table 16.

Using the mean difference in number of seeds produced by stored pollen as compared with fresh pollen as a measure of the effect of storage on vitality, it is seen that the stored pollen on an average produced approximately 17 more seeds per ear (actually 17.04 seeds) than the fresh pollen. However, the factors other than storage affecting the number of seeds are so numerous that it is extremely questionable, with only 24 pairs, whether the difference in number of seeds can be utilized as an indication of the condition of the pollen. The observed difference indicates an increase in viability of 5.6 per cent.

It is seen that the percentage of waxy seeds is increased in those ears resulting from the application of stored pollen when compared with ears resulting from the duplicate samples of fresh pollen. The difference is 6.14 ± 1.07 per cent and may be compared with that of 9.35 ± 1.26 found in the series shown in Table 16. Although the ears resulting from pollen stored, on an average, 6 hours and 52 minutes have a lower percentage than those obtained with pollen stored for 7 hours and 9 minutes the difference of 3.21 ± 1.65 per cent between the two sets can be attributed to chance, making it unnecessary to assume that a difference of 17 minutes in the period of storage results in such a large alteration in the proportion of waxy-to horny-bearing gametes.

Having seen that the effect of storage on the proportion of functioning waxy gametes is similar for the two series, the effect on the percentage of white may be examined. The data show that in this progeny where waxy endosperm and colored aleurone are associated in inheritance the percentage of white seeds resulting from the use of stored pollen is less than that from fresh pollen. This condition is in accord with the hypothesis that whatever selective force is operative on the stored gametes effects directly only the gametes bearing the waxy gene. In this case the change produced in the proportion of white to colored seeds is but a secondary effect due to the genetic correlation of waxy with the factor for colored aleurone.

The change observed in the percentage of white is significant statistically, and in amount accords fairly well with the percentage expected from the regression of white on waxy. Thus the regresssion of white on waxy in the fresh pollen was -0.305 and in the stored pollen -0.155. Using the figure for the fresh pollen as a constant from which to calculate the percentage of white seeds expected to follow a change of 6.14 per cent in the waxy, it is found that the percentage of white seeds in the stored pollen should be 1.87 less than that in the fresh pollen, whereas it was 3.43 ± 0.89 per cent less. The difference, however, though large is not significant.

EFFECT OF POLLEN STORAGE WHERE ONLY Wx IS HETEROZYGOUS

There are 24 pairs of ears that resulted from crosses where plants heterozygous for *Wx* and homozygous for *c* were used as male parents on plants homozygous for *wx* and heterozygous for *C*. In these cases a period of storage can affect only the proportion of waxy to nonwaxy since the male gametes were all of one kind with respect to color, namely, homozygous for *c*. The data for these ears are shown in Table 18. The plants bearing these ears were sibs of the plants producing the 44 pairs shown in Table 16.

The mean period of storage for the pollen used on 24 pairs of ears for which data are given in Table 18 was 6 hours and 20 minutes and the ears from stored pollen had on an average 34.1 less seeds than those from fresh pollen, an indicated death rate of 10.2 per cent.

As in the previous two cases, the percentage of waxy seeds on ears resulting from stored pollen is greater than on ears produced from fresh pollen, the excess in this instance being 7.13 ± 2.48 per cent. No difference is expected in the ratio of white to colored seeds and none was found.

A similar set of pollinations was made in the progeny where *C* and *wx* were linked and 10 successful pairs were obtained. The plants from which these ears came where sibs of the plants shown in Table 17, but differed from them in that the female parents were constituted *C c wx wx* and the male parents had the gametic constitution *c c Wx wx*. In these cases, therefore, the storage of pollen could affect only the proportion of waxy to nonwaxy, and the percentage of white seeds should be alike on the ears from fresh and stored pollen.

The data are shown in Table 19.

In full agreement with expectation there was no difference between the ears resulting from fresh pollen and those obtained with stored pollen in the percentage of white seeds; the difference being 0.76 ± 0.85 per cent.

In the case of the waxy-nonwaxy ratio, however, it is seen that the ears resulting from stored pollen have on an average 4.30 ± 1.47 per cent more waxy seeds than those obtained with fresh pollen.

With these ears the period of storage averaged six hours and six minutes, the shortest period for any of the four groups studied, and the effect on the waxy ratio is less than in the other three groups. The ears resulting from stored pollen had on an average 126 seeds less than those obtained with fresh pollen, indicating a decrease in viability of the stored gametes, of 28 per cent.

TABLE 18.—*Comparison of ears resulting from fresh and stored pollen (C c wx wx ♀ × cc Wx wx ♂); no linkage is involved in these ears (progeny Dh 416 L3 L1 C5 L4 L1 L1 L25)*

Date	Fresh pollen							Stored pollen						Differences				
	Time, a. m.	Female No.	Male No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over[a]	Time, p. m.	Female No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over	Per cent white, p. m.–a. m.	Per cent waxy, p. m.–a. m.	Per cent crossing over, p. m.–a. m.	Time,[b] p. m.–a. m.	Seeds, a. m.–p. m.
Aug. 22	9. 15	1743	1821	335	47. 2±1. 83	54. 0±2. 66	44. 2±1. 83	5. 90	1743–2	59	59. 3±4. 31	94. 9±1. 93	54. 1±4. 36	12. 1±4. 68	40. 9±3. 28	9. 9±4. 73	7. 45	276
Aug. 14	11. 00	1745	1813	56	58. 9±4. 42	35. 7±5. 60	40. 9±5. 76	5. 05	1745–2	310	51. 3±1. 91	44. 5±1. 90	46. 6±1. 90	−7. 6±4. 82	8. 8±5. 92	5. 7±6. 06	6. 05	−254
Aug. 15	11. 15	1746	1801	501	51. 9±1. 50	46. 9±1. 49	52. 8±1. 49	4. 40	1746–2	436	51. 1±1. 61	56. 4±1. 60	48. 5±1. 61	−. 8±2. 19	9. 5±2. 19	−4. 3±2. 19	5. 25	65
Aug. 17	9. 35	1747	1827	490	53. 3±1. 51	44. 7±1. 51	46. 5±1. 52	5. 05	1760	18	61. 1±7. 75	77. 8±2. 08	60. 0±7. 78	7. 8±7. 89	33. 1±2. 57	13. 5±7. 92	7. 30	472
Aug. 19	10. 35	1770	1814	154	51. 3±2. 72	49. 5±2. 72	51. 3±4. 99	5. 05	1747	261	49. 0±2. 08	57. 8±2. 06	46. 9±2. 08	−2. 3±3. 42	8. 3±3. 41	−4. 4±5. 40	6. 30	−107
Aug. 15	11. 15	1748	1810	573	49. 4±1. 40	46. 6±1. 40	48. 2±1. 40	4. 35	1749	519	51. 0±1. 48	52. 2±1. 48	51. 3±1. 48	1. 6±2. 05	5. 6±2. 05	3. 1±2. 05	5. 20	54
Aug. 19	10. 35	1750	1833	110	48. 2±3. 21	40. 9±3. 16	49. ₄±3. 21	5. 05	1750–2	340	51. 5±1. 82	58. 8±1. 79	46. 4±1. 82	3. 3±3. 69	17. 9±3. 63	−3. 0±3. 69	6. 30	−230
Aug. 15	11. 30	1751	1812	511	49. 9±1. 49	51. 3±1. 49	49. 5±1. 49	4. 35	1753	460	50. 0±1. 57	52. 0±1. 57	52. 4±1. 57	. 1±2. 17	. 7±2. 17	2. 9±2. 17	5. 05	51
Aug. 16	9. 35	1763–2	1837	502	45. 4±1. 49	45. 6±1. 49	47. 6±1. 50	4. 40	1751–2	51	51. 0±4. 71	64. 7±4. 51	57. 9±4. 66	5. 6±4. 84	19. 1±4. 75	10. 3±4. 90	7. 05	451
Aug. 15	11. 20	1758	1843	258	48. 1±2. 09	50. 4±2. 10	52. 7±2. 09	4. 35	1758–2	435	49. 0±1. 61	49. 4±1. 61	49. 9±1. 61	. 9±2. 65	−1. 0±2. 65	−2. 8±2. 65	5. 15	−177
Aug. 14	10. 45	1789	1845	627	49. 5±1. 34	42. 1±1. 33	51. 8±1. 34	5. 05	1763	520	49. 2±1. 47	45. 0±1. 47	49. 9±1. 48	−. 3±2. 00	2. 9±1. 98	−1. 9±2. 00	6. 20	107
Aug. 15	11. 30	1766	1802	576	52. 1±1. 40	46. 4±1. 40	47. 9±1. 40	4. 35	1771	257	55. 6±2. 08	49. 0±2. 10	47. 7±2. 09	3. 5±2. 51	2. 6±2. 53	−. 2±2. 51	5. 05	319
Aug. 16	9. 40	1767	1803	447	52. 1±1. 59	44. 3±1. 58	48. 3±1. 59	4. 45	1767–2	448	50. 7±1. 59	52. 9±1. 58	48. 2±1. 59	−1. 4±2. 26	8. 6±2. 24	−. 1±2. 26	7. 05	−1
Aug. 19	10. 35	1769–2	1810	82	58. 5±3. 66	54. 9±3. 70	55. 9±3. 69	5. 10	1770	447	48. 5±1. 59	44. 1±1. 58	47. 1±1. 59	−10. 0±3. 99	−10. 8±4. 02	−8. 8±4. 03	6. 35	−365
Aug. 14	11. 00	1772	1837	117	57. 3±3. 08	53. 8±3. 10	44. 9±3. 09	5. 05	1772–2	453	47. 5±1. 57	45. 9±1. 57	52. 1±1. 58	−9. 8±3. 46	−7. 9±3. 48	7. 2±3. 46	6. 05	−336
Aug. 22	9. 20	1773	1840	269	52. 4±2. 04	42. 8±2. 03	53. 3±2. 04	5. 00	1773–2	366	47. 8±1. 76	59. 8±1. 72	48. 1±1. 76	−4. 6±2. 70	17. 0±2. 67	−5. 2±2. 70	7. 40	−97
Aug. 19	10. 35	1774	1842	53	47. 2±4. 60	47. 2±4. 60	49. 2±4. 63	5. 10	1782	85	50. 6±3. 66	67. 0±3. 44	36. 1±3. 50	3. 4±5. 88	19. 8±5. 74	−13. 1±5. 81	6. 35	−32
Aug. 15	11. 25	1775	1808	426	49. 5±1. 63	46. 5±1. 62	46. 2±1. 63	4. 35	1775–2	255	47. 4±2. 10	52. 9±2. 10	47. 7±2. 10	−2. 1±2. 66	6. 4±2. 65	1. 5±2. 67	5. 10	171
Aug. 17	9. 45	1779	1831	395	46. 6±1. 68	47. 8±1. 69	49. 0±1. 69	5. 10	1779–2	318	50. 0±1. 89	54. 4±1. 88	51. 0±1. 89	3. 4±2. 53	6. 6±2. 53	2. 0±2. 53	7. 25	77
Aug. 15	11. 30	1780	1831	658	47. 7±1. 31	47. 1±1. 31	50. 6±1. 31	4. 35	1781	411	50. 1±1. 66	48. 9±1. 65	48. 9±1. 66	2. 4±2. 12	1. 8±2. 10	−1. 7±2. 12	5. 05	247
Aug. 19	10. 35	1782–2	1838	185	45. 4±2. 46	40. 0±2. 41	42. 7±2. 45	5. 10	1783–2	22	50. 1±7. 18	36. 4±6. 90	40. 1±7. 02	4. 7±2. 40	−3. 6±7. 30	−2. 6±7. 44	6. 35	163
Aug. 14	10. 50	1786	1841	320	48. 1±1. 88	49. 7±1. 88	46. 6±1. 88	5. 05	1786–2	64	56. 2±4. 19	28. 1±3. 78	37. 9±4. 08	8. 1±4. 59	−21. 6±4. 22	−8. 7±4. 49	6. 15	256
Do	10. 45	1788	1844	172	45. 3±2. 55	43. 0±2. 54	45. 9±2. 56	5. 05	1788–2	229	48. 0±2. 22	48. 0±2. 22	52. 5±2. 23	2. 7±3. 38	5. 0±3. 38	6. 6±3. 39	6. 20	−57
Aug. 17	9. 40	1790–2	1802	181	47. 0±2. 50	49. 7±2. 50	46. 4±2. 50	5. 05	1790	415	52. 5±1. 64	50. 6±1. 65	50. 6±1. 65	5. 5±2. 98	. 9±3. 00	4. 2±3. 00	7. 25	−234
Mean				333	49. 6±2. 31	46. 7±2. 55	48. 8±2. 39			299	50. 2±2. 00	51. 5±3. 63	49. 2±2. 32	. 49±. 56	7. 13±2. 48	. 005± . 61	6. 20	34

[a] No crossing over is possible in crosses of this nature. This column gives the percentage of crossovers as calculated from the four classes of seed.
[b] Time is given in hours and minutes.

TABLE 19.—*Comparison of ears resulting from fresh and stored pollen (C c wx wx ♀ × c c Wx wx ♂), no linkage is involved in these ears (progeny Dh 416 L3 L1 C5 L4 L1 L2 L25)*

	Fresh pollen							Stored pollen						Differences				
Date	Time, a. m.	Female No.	Male No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over[a]	Time, p. m.	Female No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over	Per cent white, p. m.–a. m.	Per cent waxy, p. m.–a. m.	Per cent crossing over, p. m.–a. m.	Time,[b] p. m.–a. m.	Seeds, a. m.–p. m.
Aug. 15	11.00	1968	2008	134	42.5±2.87	46.3±2.90	44.8±2.89	4.50	1968–2	460	49.8±1.57	45.4±1.56	52.6±1.57	7.3±3.27	−.9±3.28	7.8±3.28	5.50	−326
Aug. 19	10.45	1981	2022	396	46.5±1.68	44.7±1.68	49.8±1.69	5.20	1969	329	46.8±1.85	50.4±1.86	50.8±1.86	.3±2.50	5.7±2.50	1.0±2.51	6.35	67
Aug. 16	9.55	1983	2044	373	45.7±1.52	43.4±1.73	39.1±1.70	5.00	1974	360	44.4±1.52	64.4±1.70	40.1±1.74	−1.3±2.15	21.0±2.42	1.0±2.43	7.05	13
Aug. 15	10.50	1976	2016	523	48.8±1.46	43.2±1.45	51.1±1.47	4.50	1985	402	49.0±1.67	46.8±1.67	51.4±1.68	.2±2.29	3.6±2.21	.3±2.24	6.00	121
Aug. 14	11.35	1977	2046	369	52.6±1.75	43.9±1.74	50.4±1.75	5.25	1977–2	44	38.6±4.95	47.7±5.06	54.3±5.05	−14.0±5.24	3.8±5.35	3.9±5.34	5.50	325
Aug. 16	9.55	1987	2042	256	53.5±2.09	46.5±2.10	46.3±2.10	4.55	1984	497	48.1±1.51	50.3±1.51	49.1±1.51	−5.4±2.58	3.8±2.58	2.8±2.58	7.00	−241
Aug. 14	11.30	1986	2045	637	48.0±1.36	42.7±1.34	45.9±1.33	5.20	1986–2	93	46.2±3.48	45.2±3.35	57.8±3.45	−1.8±3.74	2.5±3.61	11.9±3.70	5.50	544
Aug. 15	10.50	1989	2039	840	50.8±1.16	47.3±1.16	49.3±1.16	4.50	1987	441	47.6±1.59	48.8±1.60	52.1±1.60	−3.2±1.97	1.5±1.97	2.8±1.98	6.00	399
Aug. 14	11.30	1988	2041	398	47.0±1.68	45.0±1.68	45.9±1.68	5.20	1988–2	342	50.0±1.82	44.7±1.80	49.1±1.82	3.0±2.47	−.3±2.46	3.2±2.48	5.50	56
Aug. 15	10.45	1991	2048	570	52.8±1.40	46.5±1.40	50.0±1.41	4.50	1990–2	270	53.7±2.04	46.3±2.04	46.9±2.04	.9±2.47	−.2±2.47	−3.1±2.48	6.05	300
Mean				450	49.3± .63	45.0± .39	47.8± .76			324	48.3± .60	49.4±1.31	49.6± .92	−.76±.85	4.3±1.47	2.24± .76	6.06	126

[a] No crossing over is possible in crosses of this nature. This column gives the percentage of crossovers as calculated from the four classes of seeds.
[b] Time is given in hours and minutes.

There can be little doubt that the storage of pollen, heterozygous for *Wx*, for periods of several hours, increases the proportion of functioning gametes bearing the *wx* gene.

As a means of determing how the varying periods of time used, affected the percentage of waxy seeds obtained, the correlation of time and percentage may be examined. The coefficient for the 68 sister ears shown in Tables 16 and 18 is found to be 0.325. This is not a very close relationship, but the regression of percentage on time is found to be 4.04. Since percentage distributions are being dealt with the regressions certainly are not linear but as a very general indication of the effect of time the regression will serve. It is apparent however, that on this basis, if the pollen were stored for 18 hours, all the resulting seeds would be waxy.

A very similar measure of the same thing is obtained with the coefficient of correlation between time and the magnitude of the difference between fresh and stored pollen. Here the coefficient is 0.346 and the regression of difference on time is 5.37, indicating that with each change of one hour in period of storage there is a corresponding change of 5 per cent in the difference between fresh and stored pollen.

POLLEN STORAGE INCREASES THE PROPORTION OF GAMETES BEARING THE ALEURONE COLOR FACTOR *c*

Having seen that the proportion of white to colored seeds differs in stored and fresh pollen when the waxy gene is involved, and, further, having indirect evidence that the disturbance is due to the effect of storage on the gametes bearing the *wx* gene, it may be well to examine the data accumulated from sister plants where only the *C c* gene is involved.

These data consist of ears resulting from crosses of the nature *c c Wx wx* ♀ × *C c wx wx* ♂ and were obtained from two progenies. In crosses of this nature four classes of seeds are obtained, but since the male gametes were all of one kind with respect to waxy, namely, *wx*, there can be no differential effect of storage operating upon this gene. The results are shown in Tables 20 and 21.

The percentage of waxy should be alike in both morning and afternoon pollinations, and such is the case. It is of interest to observe that the percentage very closely approximates the expected 50 in both arrays.

The difference in percentage of white in Table 20 is 1.82 ± 0.57, and for the ears in Table 21 is 1.87 ± 0.74. In neither case is the difference significant in comparison with its probable error, but in both instances the percentage of white seeds is higher on those ears resulting from stored pollen. Combining the two progenies, the mean difference becomes 1.84 ± 0.29, clearly a significant difference, showing that the storage of pollen tends to increase the proportion of functioning gametes bearing the *c* aleurone factor.

The observed changes in the proportions of white and colored seeds resulting from stored pollen were attributed entirely to the linkage between the genes *c* and *wx*. This conclusion seemed justified since the actual percentage of white seeds agreed closely with the percentage predicted from the regressions of white on waxy.

TABLE 20.—*Comparison of ears resulting from fresh and from stored pollen (c c Wx wx ♀ × C c wx wx ♂); no linkage is involved in these ears (progeny Dh 416 L3 L1 C5 L4 L1 L1 L25)*

Date	Fresh pollen							Stored pollen						Differences				
	Time, a. m.	Female No.	Male No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over [a]	Time, p. m.	Female No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over	Per cent white, p. m.–a. m.	Per cent waxy, p. m.–a. m.	Per cent crossing over, p. m.–a. m.	Time, [b] p. m.–a. m.	Seeds, a. m.–p. m.
Aug. 15	11.15	1801	1746	514	48.0±1.48	50.4±1.48	49.4±1.48	4.40	1801–2	386	48.2±1.71	51.3±1.71	52.3±1.70	0.2±2.26	0.9±2.26	2.9±2.25	5.25	128
Do	11.30	1802	1771	569	50.3±1.41	48.8±1.41	51.7±1.42	4.45	1802–2	557	50.8±1.43	54.8±1.42	52.2±1.43	.5±2.01	6.0±2.00	.5±2.02	5.15	12
Aug. 16	9.40	1803	1757	379	48.0±1.73	51.4±1.73	51.9±1.73	4.45	1804	361	51.2±1.77	44.3±1.76	51.7±1.76	3.2±2.47	−7.1±2.46	−.2±2.47	7.05	18
Aug. 17	9.45	1803–2	1742	159	49.0±2.67	49.0±2.67	47.8±2.68	5.10	1807–2	174	70.7±2.32	41.4±2.50	56.9±2.53	21.7±3.54	−7.6±3.66	9.1±3.68	7.25	−15
Aug. 16	9.35	1810–2	1751	324	48.4±1.87	50.6±1.87	50.3±1.88	4.45	1807	351	50.4±1.79	49.0±1.79	52.2±1.79	2.0±2.59	−1.6±2.59	1.9±2.59	7.10	−27
Aug. 15	11.25	1808	1775	323	48.9±1.87	48.9±1.87	53.9±1.86	4.45	1808–2	316	48.7±1.89	49.4±1.89	50.6±1.89	−.2±2.66	.5±2.66	−3.8±2.65	5.20	7
Aug. 19	10.40	1809	1770	63	47.6±4.23	50.8±4.25	47.6±4.23	5.10	1814	484	49.8±1.53	47.9±1.53	46.9±1.53	2.2±4.50	−2.9±4.51	−.7±4.50	6.30	−421
Aug. 15	11.15	1810	1748	342	52.0±1.82	50.3±1.82	47.4±1.82	4.45	1827	511	52.6±1.48	49.9±1.49	50.9±1.49	.6±2.35	−.4±2.35	3.5±2.35	5.30	−169
Do	11.30	1812	1751	392	50.8±1.70	47.4±1.70	46.6±1.69	4.45	1812–2	36	69.4±5.18	61.1±5.48	59.2±5.50	18.6±5.44	13.7±5.74	12.6±5.75	5.15	356
Aug. 14	11.05	1813	1745	644	52.5±1.32	52.3±1.32	51.8±1.34	5.05	1813–2	643	49.8±1.33	48.8±1.32	50.2±1.33	−2.7±1.87	−3.5±1.87	−1.6±2.89	6.00	1
Aug. 19	10.40	1814–2	1782	280	49.3±2.00	51.1±2.05	53.9±2.00	5.10	1827–2	14	57.1±8.90	42.8±8.90	25.7±7.86	7.8±9.12	−8.3±9.11	−28.2±8.10	6.30	266
Aug. 22	9.30	1820–2	1741	241	44.0±2.15	50.6±2.16	53.1±2.16	5.00	1820	48	39.6±4.75	50.0±4.85	43.4±4.82	−4.4±5.21	−.6±5.32	−9.7±5.28	7.30	193
Aug. 14	10.50	1826	1776	461	47.9±1.56	50.1±1.57	49.9±1.57	5.10	1826–2	327	52.6±1.86	51.4±1.86	52.7±1.85	4.7±2.43	1.3±2.43	2.8±2.42	6.20	134
Aug. 17	9.50	1828	1753	265	49.0±2.06	50.6±2.07	47.6±2.06	5.10	1839	384	50.3±1.72	51.8±1.72	48.4±1.72	1.3±2.68	1.2±2.69	.8±2.68	7.20	−119
Aug. 22	9.35	1829	1782	403	46.6±1.67	54.3±1.67	49.6±1.68	5.00	1829–2	119	52.1±3.09	47.0±3.07	58.8±3.04	5.5±3.51	−7.3±3.50	9.2±3.47	7.25	284
Aug. 15	11.30	1831	1780	220	45.9±2.26	46.8±2.26	47.5±2.26	4.45	1831–2	336	45.8±1.83	49.7±1.83	48.5±1.83	−.1±2.91	2.9±2.91	1.0±2.91	5.15	−116
Do	11.30	1834	1781	623	49.6±1.35	50.2±1.34	51.1±1.35	4.50	1834–2	177	52.0±2.52	53.1±2.52	49.8±2.53	2.4±2.86	2.9±2.86	−1.3±2.87	5.20	446
Aug. 14	11.00	1837	1772	689	50.2±1.28	47.0±1.28	49.3±1.28	5.10	1837–2	319	53.6±1.88	52.4±1.88	47.2±1.88	3.4±2.27	5.4±2.28	−2.1±2.27	6.10	370
Aug. 16	9.30	1838	1785	225	53.3±2.23	48.9±2.24	48.8±2.24	4.45	1838–2	148	56.1±2.74	47.3±2.76	47.6±2.76	2.8±3.53	−1.6±3.55	−1.2±3.56	7.15	77
Aug. 14	10.50	1841	1786	725	49.1±1.25	51.6±1.25	46.8±1.24	5.10	1841–2	139	49.6±2.85	48.2±2.85	47.5±2.84	.5±3.11	−3.4±3.11	.7±3.10	6.20	586
Aug. 19	10.40	1842	1783	331	48.6±1.88	46.1±1.87	43.1±1.86	5.10	1842–2	299	47.5±1.94	47.8±1.95	53.3±1.94	−1.1±2.70	1.7±2.70	10.2±2.69	6.30	32
Aug. 15	11.20	1843	1758	278	54.7±2.01	45.7±2.01	50.4±2.02	4.50	1843–2	247	55.9±2.06	50.2±2.14	51.1±2.14	1.2±2.88	4.5±2.94	.7±2.95	5.30	31
Aug. 14	10.45	1844	1788	336	46.4±1.83	47.6±1.83	51.4±1.83	5.10	1844–2	262	49.2±2.08	53.0±2.07	50.4±2.08	2.8±2.77	5.4±2.76	−1.0±2.77	6.25	74
Do	10.40	1845	1789	576	48.8±1.40	51.9±1.40	53.4±1.40	5.15	1845–2	622	49.5±1.35	50.3±1.35	48.1±1.35	.7±1.95	−1.6±1.94	−5.3±1.94	6.35	−46
Mean				390	49.3± .29	49.9± .29	49.9± .35			302	51.0± .57	49.9± .39	50.4± .38	1.82± .57	.29± .56	.54± .61	6.11	88

[a] In crosses of this nature no crossing-over is possible. This column gives the percentage of crossovers as calculated from the four classes of seeds.
[b] Time is given in hours and minutes.

TABLE 21.—*Comparison of ears resulting from fresh and stored pollen (c c Wx wx ♀ × C c wx wx ♂); no linkage is involved in these ears (progeny Dh 416 L3 L1 C5 L4 L1 L2 L25)*

Date	Fresh pollen							Stored pollen						Differences				
	Time, a. m.	Female No.	Male No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over [a]	Time, p. m.	Female No.	Total seeds	Per cent white	Per cent waxy	Per cent crossing over	Per cent white, p. m.–a. m.	Per cent waxy, p. a.–a. m.	Per cent crossing over, p. m.–a. m.	Time, [b] p. m.–a. m.	Seeds, a. m.–p. m.
Aug. 17	10.00	2001	1970	342	49.1±1.82	55.6±1.80	51.6±1.83	5.15	2006-2	241	51.4±2.16	49.4±2.16	46.5±2.16	2.3±2.82	−6.2±2.81	−5.1±2.83	7.15	101
Aug. 15	11.00	2004	1970	110	46.4±3.19	42.7±3.18	48.5±3.21	5.00	2004-2	111	52.2±3.19	52.2±3.19	48.8±3.20	5.8±4.51	9.5±4.52	.3±4.53	6.00	−1
Do	11.00	2006	1976	157	42.7±2.66	51.6±2.70	50.5±2.69	4.55	2019	244	49.6±2.15	48.0±2.16	46.7±2.15	6.9±3.42	−3.6±3.46	−3.8±3.44	5.55	−87
Aug. 19	10.50	2003–2	1981	249	51.0±2.14	51.2±2.12	47.8±2.13	5.30	2007	227	44.0±2.22	47.1±2.23	49.0±2.24	−7.0±3.08	−4.1±3.08	1.2±3.09	6.40	22
Aug. 15	11.00	2008	1968	633	47.4±1.33	49.1±1.34	52.7±1.33	5.00	2008-2	598	49.7±1.38	44.5±1.37	52.3±1.37	2.3±1.92	−4.6±1.92	−.4±2.21	6.00	35
Do	10.55	2016	1985	641	50.1±1.32	48.5±1.33	47.6±1.32	4.55	2016-2	524	48.7±1.47	49.0±1.47	49.6±1.47	−1.4±1.98	.5±1.98	2.0±1.97	6.00	117
Do	11.00	2017	1974	231	42.4±2.18	51.9±2.22	52.7±2.20	4.55	2019-2	622	51.4±1.35	46.6±1.35	49.6±1.35	9.0±2.56	−5.3±2.60	−3.1±2.58	5.55	−391
Do	10.50	2021	1987	687	50.4±1.28	51.7±1.28	48.9±1.28	4.55	2021-2	675	56.4±1.29	49.0±1.29	50.9±1.30	6.0±1.82	−2.7±1.82	2.0±1.83	6.05	12
Aug. 17	10.00	2022	1974	165	43.6±2.60	46.7±2.61	49.3±2.62	5.15	2022-2	186	53.2±2.46	51.1±2.47	48.3±2.46	9.6±3.58	4.4±3.60	−1.0±3.60	7.15	−21
Aug. 16	9.50	2024	1974	97	42.3±3.38	45.4±3.41	38.0±3.32	4.55	2035	412	50.7±1.66	45.9±1.65	53.5±1.65	8.4±3.76	.5±3.79	15.5±3.57	7.05	−315
Aug. 17	10.05	2029	1983	234	46.6±2.19	48.7±2.20	46.5±2.19	5.15	2032	419	51.1±1.65	54.4±1.64	51.7±1.65	4.5±2.74	5.7±2.74	5.2±2.74	7.10	−185
Aug. 15	10.50	2039	1989	591	46.2±1.38	53.1±1.38	52.0±1.38	4.50	2034	273	54.6±2.03	49.8±2.04	48.3±2.04	8.4±2.46	−3.3±2.46	−3.7±2.46	6.00	318
Aug. 16	9.50	2036	1985	91	50.5±3.34	46.2±3.35	47.3±3.52	4.55	2042	243	51.0±2.16	45.7±2.15	47.8±2.16	.5±3.98	−.5±3.98	.5±4.13	7.05	−152
Aug. 14	11.25	2041	1988	576	54.5±1.43	54.5±1.40	52.7±1.40	5.30	2041-2	322	49.1±1.87	49.7±1.87	50.3±1.88	−5.4±2.35	−4.8±2.34	−2.4±2.34	6.05	254
Aug. 15	10.45	2048	1991	736	49.0±1.23	49.3±1.24	47.8±1.24	4.50	2043	311	51.4±1.91	43.4±1.89	53.7±1.90	2.4±2.27	−5.9±2.26	5.9±2.27	6.05	425
Aug. 19	10.50	2058	1983	556	50.9±1.43	50.2±1.43	53.6±1.45	5.30	2053-2	623	51.4±1.35	48.2±1.35	52.8±1.35	.5±1.96	−2.0±1.96	−.8±1.98	6.40	−67
Aug. 15	11.10	2055	1986	640	49.2±1.33	51.6±1.33	48.6±1.33	5.00	2055-2	104	44.2±3.27	57.7±3.26	52.9±3.30	−5.0±3.53	6.1±3.52	4.3±3.56	5.50	536
Do	11.10	2056	1984	428	53.5±1.62	45.6±1.62	50.3±1.63	5.00	2058	439	48.5±1.61	51.7±1.60	50.4±1.61	−5.0±2.28	6.1±2.28	.1±2.29	5.50	−11
Do	11.10	2062	1983	252	51.2±2.12	48.4±2.12	46.4±2.12	5.05	2062-2	337	50.4±1.83	50.7±1.83	47.4±1.83	−.8±2.80	2.3±2.80	1.0±2.80	5.55	−85
Mean	------	------	------	390	49.2± .48	50.4± .44	49.9± .43	------	------	364	50.9± .43	48.6± .47	50.4± .33	1.87± .74	−1.32± .64	.71± .59	6.19	26

[a] No crossing over is possible in crosses of this nature. This column gives the percentage of crossovers as calculated from the four classes of seeds.
[b] Time is given in hours and minutes.

Thus in the progeny where the linkage is between *C* and *wx* the regression of percentage white on percentage waxy is -0.305. An increase of 6.14 in the percentage of waxy seeds should be accompanied, therefore, by a decrease of 1.87 in the percentage of white seeds. Actually the observed decrease was 3.43 ± 0.89 per cent, not a bad agreement with expectation, and the error would permit of an actual difference of 0.76.

Assuming that the period of storage used on the plants shown in Table 17 had affected the gametes bearing the *c* gene to the same extent as was observed to be the case for the plants in Tables 20 and 21, then the difference in percentage white on ears resulting from the use of stored and fresh pollen should have been 0.03 per cent with the percentage of white in the stored pollen less than that of the fresh. This figure is well within the error of the observed difference and the agreement with the figure obtained by use of the regression coefficient may be entirely misleading.

POLLEN STORAGE AFFECTS THE CROSSOVER RATIO OF *C* AND *Wx*

Without exception, where due allowance is made for differences in time of storage and the observed increases corrected for the regression of difference on time, there is found to be a greater effect of storage on both *c* and *wx* when these factors are considered separately.

The data do not provide material for testing the effect of storage on the ratios of one pair of allelomorphs when the other pair is homozygous dominant, since in all instances where only one pair is heterozygous the other is homozygous recessive. This is a misfortune since it means that in those cases where the effect of storage on a single gene is measured it is in reality the effect of storage upon the double recessive combination *c wx*.

In all of the comparisons the effect of storage apparently increases both the *c* and *wx* genes to a greater extent when only one of the pair is heterozygous than when both are heterozygous, indicating that the selective action is more pronounced upon the double recessive combination than would be expected from the degree of selection found for the two genes separately.

This condition necessitates an alteration in the linkage between these two genes in the stored as compared with the fresh pollen. In the 13 ears reported by Collins and Kempton (*5*) it was concluded that storage failed to alter the crossover rate, or at least the effect was not greater than 2 per cent.

From the comparison of the effect of storage where *c* and *wx* are heterozygous with that found for these two factors separately, an alteration in the crossover rate approximating 2 per cent would be expected. Where the genes *c* and *wx* are linked any selective force that operated in such a manner as to increase the proportion of the gametes bearing the double recessive combinations would reduce the number of crossovers. Conversely, when *C* and *wx* are linked a selective action that tended to increase both *c* and *wx* should increase the number of crossovers. An examination of Tables 16 and 17 shows this to be the case. As a result of pollen storage, crossing over is reduced in the group of ears where *c* and *wx* are known to be linked and is increased when *C* and *wx* are linked. In neither instance is the difference large, but when the two differences are compared the

difference between them is 3.73 ± 0.95, a figure exceeding three times the error and probably significant.

This evidence, though slight, tends to confirm the conclusion that the combination of the genes *c* and *wx* is operated upon by some selective agent when the opportunity is presented by storing the pollen for several hours.

GAMETIC RATIOS UNALTERED IN POLLEN STORED OVER-NIGHT

Pollen from three heterozygous plants was collected in the afternoon, and approximately one-half of each lot was applied immediately to homozygous recessive plants. The remaining halves were stored over-night in the collection bags and applied the following morning to other homozygous recessive plants. The time of storage varied from 14 to 16 hours. Only a few such comparisons were made since it was believed to be very doubtful whether the pollen would remain viable, and the number of plants available for experimentation was limited.

The morning following storage the pollen was very unpromising in appearance, but nevertheless seeds were obtained on the three ears resulting from its use. The data are shown in Table 22.

In only two of the three cases was the percentage of waxy seeds obtained from the use of this stored pollen higher than that obtained with fresh pollen. The ears resulting from stored pollen are so small as to make the definite determination of any but very large differences impossible.

The data are presented here simply because it indicates that these long periods of storage at night do not bring about correspondingly large increases in the proportion of waxy to nonwaxy gametes.

TABLE 22.—*Comparison of ears resulting from the use of fresh and stored pollen; pollen collected from the plants in the afternoon, one-half applied immediately to double recessive plants, the other stored overnight and applied the following morning*

Plant No. (female)	Plant No. (male)	Pollen collected	Pollen applied	Number of seeds	Per cent white	Per cent waxy	Per cent crossing over
3642	3986	5.20 p. m.	5.20 p. m.	654	49.7±1.32	48.3±1.32	37.7±1.28
3642–2	3986	5.20 p. m.	7.45 a. m.	191	45.0±2.42	48.2±2.43	35.6±2.34
Difference		14 hours 25 minutes		463	−4.7±	−.1	−2.1
1593–2	1881	4.20 p. m.	4.20 p. m.	520	51.5±1.96	48.5±1.48	35.7±1.41
1612	1881	4.20 p. m.	8.45 a. m.	61	50.0±2.40	49.5±2.40	38.2±2.34
Difference		16 hours 25 minutes		459	−1.5	1.0	2.5
1624	1876	4.30 p. m.	4.30 p. m.	151	50.3±2.74	45.0±2.72	36.9±2.64
1594	1876	4.30 p. m.	8.40 a. m.	56	41.1±4.40	51.8±4.50	38.5±4.39
Difference		16 hours 10 minutes		95	−9.2	6.8	1.6

It should be borne in mind that this pollen was stored in the dark, thus eliminating any effect that light might have on stored pollen; though Brink's (*3*) experiments would seem to have eliminated light as a factor in the alteration of gametic ratios.

In considering these three cases it should also be borne in mind that the pollen was collected in the afternoon and the ratio of waxy to nonwaxy gametes may have receded from the high point even at the time of collection.

To this experiment may be added one other case where a tassel branch was removed from a plant and stored overnight with the lower end in water. The pollen was shaken out and applied the following morning. In the case of this plant the linkage was between *C* and *wx*. The ear obtained from this procedure may be compared with an ear resulting from the application of fresh pollen collected on the morning before the branch was removed, and also with an ear that resulted from the use of pollen collected in the morning and stored throughout that same day on the plant in the field, and, further, with a third ear resulting from the application of fresh pollen collected on the morning following the removal of the tassel branch.

The data from these ears are shown in Table 23.

TABLE 23.—*Comparison of ears resulting from the use of fresh and stored pollen with an ear obtained by the use of pollen from a tassel branch kept for 15 hours and 45 minutes in water; the source of the pollen was a single plant heterozygous for C and Wx with the linkage between C and wx*

POLLEN FROM ENTIRE TASSEL

Pollen collected	Pollen applied	Number of seeds	Per cent white	Per cent waxy	Per cent crossing over
10.35 a. m., Aug. 15	10.35 a. m., Aug. 15	178	50.0±2.52	49.4±2.53	29.7±2.30
Do	4.35 p. m., Aug. 15	519	49.9±1.48	53.4±1.46	31.5±1.37
8.30 a. m., Aug. 16	8.30 a. m., Aug. 16	347	54.5±1.80	43.5±1.80	32.7±1.69

POLLEN FROM A TASSEL BRANCH

4 p. m., Aug. 15	7.45 a. m., Aug. 16	285	41.4±1.96	50.2±2.00	30.8±1.84

The chief point of interest in this comparison is the fact that the percentage of waxy seeds on the ear resulting from pollen produced by a tassel branch stored overnight in water, is not increased above the expected 50 per cent.

TABLE 24.—*Comparison of ears resulting from the use of fresh and stored pollen with an ear obtained by the use of pollen from a tassel branch kept for 23½ hours in 50 per cent alcohol; the source of the pollen was a single plant heterozygous for C and Wx with a linkage between C and wx*

POLLEN FROM ENTIRE TASSEL

Pollen collected	Pollen applied	Number of seeds	Per cent white	Per cent waxy	Per cent crossing over
8.30 a. m., Aug. 16	8.30 a. m., Aug. 16	347	54.5±1.80	43.5±1.80	32.7±1.69
9.55 a. m., Aug. 17	9.55 a. m., Aug. 17	293	50.2±1.97	43.7±1.95	33.0±1.86
Do	5.15 p. m., Aug. 17	229	36.7±2.15	68.6±2.07	28.5±2.01

POLLEN FROM A TASSEL BRANCH

4.00 p. m., Aug. 16	3.30 p. m., Aug. 17	102	49.0±3.34	56.9±3.30	33.0±3.19

A very similar experiment was carried out with another tassel branch from this same plant, but instead of standing in water this tassel branch was put into 50 per cent alcohol and kept until 3.30

p. m. the following afternoon. The ear resulting from this treatment may be compared with an ear obtained with fresh pollen on the morning of the day the tassel branch was removed and with two other ears, one the result of fresh pollen applied in the morning after the branch was removed and the other the result of storing the pollen in the field on the day that the pollen from the removed branch was applied.

The ears are too small to permit any definite conclusions, but it is clear that the treatment accorded the tassel branch kept in alcohol did not affect the ratio of waxy to nonwaxy gametes beyond that found when stored pollen is used. The data are shown in Table 24.

DISCUSSION

Although the fact that the storage of pollen increases the proportion of functioning gametes bearing the waxy gene and also the aleurone color gene *c*, may be considered as demonstrated, there is little evidence as to what conditions, present during storage, are responsible for the observed results.

Brink's (*3*) experiments with irradiation would seem to dispose of light as a factor in the alteration of the ratios, although in his experiments the proportion of waxy gametes increased with the increase in the period of irradiation. It is possible that the phenomenon is one of partial dehydration, though Brink partially desiccated the pollen in one experiment and obtained a slight decrease in the percentage of waxy. Attempts to gain an insight into the causal factors by cooling, drying, heating, etc., were made during the past season. In all but the cooling experiments these attempts resulted in complete failure to obtain seeds. This failure, however, can not be attributed entirely to treatment as the untreated controls also were largely failures, apparently owing to the protracted drought prevalent at the time pollinations were made.

Curiously enough, the only well-fertilized ears in the entire experiment, involving some 300 pollinations, were those that resulted from the application of pollen stored in desiccators at a temperature of 50° F. for a period of approximately seven hours.

As none of the applications of fresh untreated pollen produced ears of any consequence, no control is available with which to compare the cooled stored pollen. The ears produced as the result of fertilization with such pollen have abnormal waxy ratios entirely comparable with those obtained in the preceding years with pollen stored on the plant, but without controls it is not possible to determine whether the effect of cold storage is slightly greater or less than that of field storage.

Lacking more precise information as to actual causes it may not be amiss to make provisional deductions from the data.

In presenting the evidence for the effect of pollen storage on the waxy ratio, the difference between the ears resulting from fresh pollen as compared with those obtained with stored pollen has been stressed rather than the departure of the latter group from an expected percentage. This method of treatment was necessitated by the uncertainty as to the expected proportions, since in many progenies there is consistent deficiency of functioning waxy gametes in one sex or the other.

Placing the emphasis on the difference between the two sets of ears tends to obscure the fact that those ears resulting from fresh pollen have a deficiency of waxy seeds below equality almost as pronounced as the excess of waxy seeds on the ears resulting from stored pollen. This condition is shown in Figure 1.

The observed percentage of waxy seeds on all the ears resulting from the use of fresh pollen is 46.79 ± 0.30. The departure from the expected 50 per cent is 10.7 times the error. Pollen counts by Longley (*9*) and others have shown, even in very early stages, an equality of gametes bearing waxy to those having the dominant allelomorph. It is apparent that the proportion of gametes which fail to function is greater among those bearing the waxy gene than among those bearing the dominant allelomorph.

The deficiency of functioning gametes bearing the waxy gene in fresh pollen, considered in connection with the excess of such gametes in stored pollen, points to a slower rate of maturity for such gametes rather than to a higher death rate for gametes bearing the nonwaxy gene.

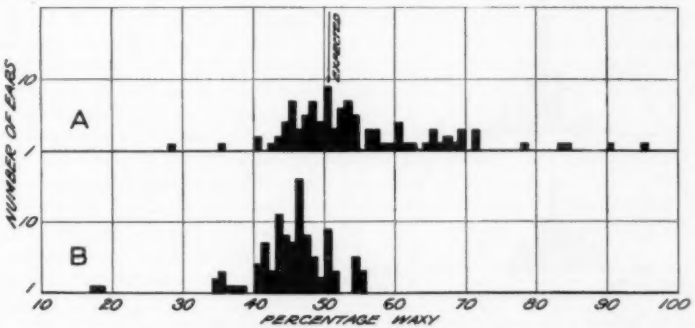

FIG. 1.—Frequency distributions of percentage of waxy seeds on ears obtained with fresh and stored pollen: A, stored pollen; B, fresh pollen

It is true that if the chemical nature of the gametes bearing waxy was such that these gametes absorbed or retained moisture, causing them to adhere to one another, a period of storage would provide time for them to become dry and to separate, thus increasing the effective gametes of this nature. Such a condition would result in restoring the ratio of waxy to nonwaxy from one of defect in fresh pollen to one of equality in stored pollen, but it would not result in increasing the ratio above equality unless accompanied by a loss of vitality of the gametes bearing the nonwaxy gene.

The simplest explanation then would seem to lie in the assumption that gametes bearing the *wx* and the *c* genes are not mature at the time of anthesis and that by the time they have attained maturity many of the gametes bearing the *Wx* or the *C* genes have lost their vitality.

Were the effect of storage restricted to the effect on the *wx* gene such an explanation might have merit since it is known that gametes bearing the *wx* gene differ chemically from those bearing *Wx*. But pollen storage seems also to have an effect on the *c* aleurone factor where a logical chemical explanation can only be surmised.

The gametes bearing the aleurone color gene *c* are not affected by storage to the same degree as those bearing the *Wx* gene, but the evidence seems conclusive that there is a tendency for such gametes to be in excess of equality in stored pollen. There is some evidence also that the functioning gametes bearing the *c* gene are less than equality in fresh pollen (fig. 2).

In calculating the mean percentage of white seeds on ears resulting from fresh pollen it has been necessary to eliminate ears that resulted from the application of pollen heterozygous for both *C* and *Wx*. In back crosses where the male parent is heterozygous for *C* and *Wx* and the linkage is between the dominant allelomorphs, a deficiency of waxy is accompanied by a deficiency of white and, conversely, if dominant and recessive genes are linked a deficiency of waxy results in an excess of colored. If such back crosses were not eliminated in calculating the mean percentage of white seeds there would result only an indirect measure of the influence of the waxy gene upon the colored-white ratios.

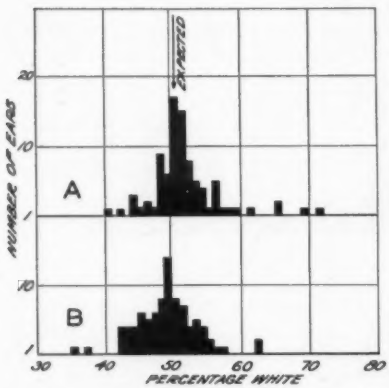

FIG. 2.—Frequency distributions of percentage of white seeds on ears obtained with fresh and stored pollen; A, stored pollen; B, fresh pollen

For all ears resulting from fresh pollen not heterozygous for both *C* and *Wx*, the mean percentage of white seeds is found to be 49.12 ± 0.29, a departure from 50 per cent, just exceeding three times the error.

This result does not furnish conclusive proof that the ratio of gametes bearing *c* to those having the dominant allelomorph *C* is less than equality in fresh pollen.

It has been seen that pollen storage affects the gametes bearing the *c* gene to a much less extent than it does gametes with the *wx* gene and apparently the *C c* pair has less influence on the differential functioning of the gametes than the *Wx wx* pair.

With the number of ears available it is not possible to determine with reasonable certainty for the colored-white ratio departures, from the expected equality, of less than 1 per cent. Until further data are available, therefore, it would be unwise to assume that functioning gametes bearing the *c* gene are below equality in fresh pollen.

That the storage of pollen will be found to have an effect on the ratios of other genes seems a reasonable prediction justified by the results obtained with *wx* and *c*.

SUMMARY

When measured by differences in individual plant reciprocals there seems to be no basis for the conclusion that the ratio of waxy to nonwaxy bearing gametes is consistently less in the male than in the female gametes. Differences between the sexes with respect to

this pair of genes are found, but in some progenies the proportion of waxy is higher in the female and in others in the male gametes.

The percentage of waxy seeds is alike on upper and lower ears in back crosses where the male parent is heterozygous for the *Wx* gene. In back crosses where the female parent is heterozygous the percentage of waxy seeds is higher on the upper ears.

Apparently there is no difference in the percentage of waxy seeds on the butts and tips of the ears.

Long styles do not seem to be associated with a low percentage of waxy seeds.

Gametes bearing the genes *su* and *wx* seem to function as perfectly as those bearing other combinations of these genes.

The storage of pollen in the daylight for a period of approximately seven hours greatly increases the proportion of functioning gametes bearing the *wx* gene.

The storage of pollen for longer periods at night seems to have little effect on the proportion of *wx* to *Wx*.

Storage of pollen increases the proportion of gametes bearing the aleurone color gene *c*, but to a less degree than is found for *wx*.

The effect of pollen storage on the genes *c* and *wx* slightly alters the crossover ratio of these two genes. When the stored pollen is from plants with *c* and *wx* linked, the crossover percentage is reduced, and where the linkage is between *C* and *wx* the percentage of crossovers is increased.

Although the storage of pollen increases the proportion of *wx* to *Wx* and of *c* to *C*-bearing gametes to above equality, the proportion of these genes in fresh pollen is below equality.

From indirect evidence it is concluded that gametes bearing either the *wx* or *c* genes mature at a slower rate than those bearing the dominant allelomorphs.

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SELECTION FOR QUALITY OF OIL IN SOY BEANS[1]

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INTRODUCTION

In addition to its manifold uses in other fields of commercial activity soy-bean oil has of late years become an important factor in the paint industry. As a drying oil for paints it has passed beyond the experimental stage. In fact it is now possible to state that linseed oil, which is relatively expensive at present, can be replaced with an appreciable amount of soy-bean oil (20 to 25 per cent) to form a satisfactory oil for paints.

The drying quality of soy-bean oil is so much lower than that of linseed oil that the soy-bean oil can not be successfully used alone. It has therefore become highly desirable to attempt to increase the drying quality of the oil extracted from soy beans, for a higher quality of soy-bean oil means a smaller proportion of linseed oil for a proper drying mixture, and consequently a more economical oil for paints.

The most logical method of attempting to increase oil quality permanently in the soy bean (*Soja max*) is by breeding. This plant is well adapted for selection methods of breeding but less so for hybridization methods. This is true primarily because of the small size of the soy-bean flower, which renders crossing operations very difficult, but on the other hand, reduces the chances for contamination by cross-pollination of insects.

Experimental evidence shows that there is less than 0.2 per cent natural cross-pollination with the types under observation (*18*)[4]. Because the soy bean is so largely self-pollinated, the isolation of relatively pure strains or pure lines is often possible. It has repeatedly been demonstrated that soy beans can be grown successfully in the Northern States. In fact, they are especially well adapted to the sandy regions of Wisconsin. Since this is true, it is important to determine whether the cooler climate of Wisconsin is favorable to the production of high quality in soy beans. The work reported herein has an important bearing on this point.

PLAN OF THE SELECTION EXPERIMENT

The experiment was begun in 1912 with a single soy-bean plant, the seeds of which were analyzed both for quality and percentage of oil. The progeny of this plant was then grown to maturity and the

[1] Received for publication Feb. 19, 1927; issued July 1, 1927. Paper No. 71 from the department of genetics, agricultural experiment station, University of Wisconsin. This contribution reports a portion of the results of a cooperative project between the department of genetics and the department of agricultural chemistry. The genetic work was inaugurated by L. J. Cole and O. Lloyd Jones in 1912 and, with the assistance of C. M. Woodworth, was carried on until the spring of 1919. At that time it was turned over to E. W. Lindstrom for continuation, and finally for completion and publication.

The chemical analyses were made under the direction of E. B. Hart, in charge of the agricultural chemistry, chiefly by E. M. Nelson, and also by J. S. Jurrgens, G. D. Williams, D. W. Smith, and R. M. Bethke.

Financial assistance for a part of the chemical determinations was afforded by grants from the Paint Manufacturers' Association of the United States.

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[3] Now associate professor of plant breeding at the University of Illinois.

[4] Reference is made by number (italic) to "Literature cited," p. 94.

seed from each individual was analyzed. From the highest and the lowest analyzing plants seeds were selected for planting the following year. These were the beginning of the high and low selection lines, respectively.

Their progeny in turn was analyzed. For each succeeding year the highest-analyzing plant of the high line was selected to continue that line. Likewise the lowest-analyzing plant of the low line was chosen for continuing the low line. This procedure was followed for each of seven years. Hence the entire progeny at the end of eight years could be traced back to the single original plant.

As a result of normal self-fertilization, this process would naturally tend to produce a pure line of beans, especially for the character being selected. At the same time other obvious characters, such as flower color, growth habit, maturity, and yield, would also tend to become uniform.

Since quality of oil is more important in paint manufacture than quantity, and since the number of chemical analyses was necessarily limited, it was decided to concentrate on one of the characters, namely, quality of oil. Consequently the high and low selections were made on the basis of the drying quality of oil. However, the quantity or percentage of oil produced by each plant was also determined in every case.

ANALYTICAL METHODS

Quality of soy-bean oil for paint manufacture depends upon its capacity for rapid drying. This means its ability to combine with atmospheric oxygen, and is dependent on the degree of unsaturation of the fatty acids that go to make up the oil. The best oil is that which is highly unsaturated, and hence combines quickly with oxygen to form a thin film over the paint.

Unsaturated oils absorbed elements other than oxygen. Iodine, for example, is very rapidly absorbed. Hence iodine can be used in determining the degree of unsaturation of oils, the amount of iodine absorbed being readily measured. The percentage of iodine absorbed by an oil is called its iodine number. This is found by various standardized methods such as those of Hübl, Hanus, or Wijs. Linseed oil has an average iodine number of 180, while commercial soy-bean oil averages less than 128. For the chemical analyses of oil quality in this experiment the method of Hübl was used.

For analytical purposes air-dried samples of 10 gm. taken at random from the harvested seed of individual plants were given to the chemist. Occasionally two or three samples from a single plant were included as a check on the chemical work. The samples were labeled with other then pedigree numbers, so as to prevent any unconscious personal equation from entering into the results. The iodine values quoted herein are believed to be accurate within the limits ± 2 and the determinations on percentage of oil to ± 0.3 per cent.

FIELD METHODS

The experimental soy beans were grown in a clay-loam soil in the genetics plots at the University of Wisconsin. Except in 1913, the soil of each new plot was inoculated with soy-bean bacteria. The plots were manured lightly and plowed yearly.

Planting was all done by hand in the latter part of May or earlier. The rows were 3 feet apart, and the individual plants were spaced exactly 2 feet apart in the row. Each row was properly staked, and when the plants were mature each was labeled with a tag bearing its pedigree number.

Usually 50 or more seeds from each selected plant were grown to maturity. As far as possible these were grown in duplicate rows planted in different parts of the plot. Notes on flower color, growth habit, pubescence, and maturity were taken for each plant. These various characters served as excellent checks on the relative purity of the strains.

Harvesting the seed was done entirely by hand, the plants being pulled or cut separately. After harvesting they were taken to the seed house and dried for a period of three weeks or longer.

The plants were then threshed by careful workers. Each plant with its tag was run through a small threshing machine especially devised for this work. The construction was such that it was practically impossible for a bean to lodge in any of the various parts and so contaminate the plant that followed.

It is firmly believed that errors of mechanical mixing of seed and erroneous labeling have been reduced to a negligible minimum, since the person directly in charge of the experiment was in close personal touch with the work at all stages. Careful note-taking on the many morphological characters has made it possible to detect any very evident mixtures in the field, since the two selection lines differed in some of these characters.

CHEMICAL COMPOSITION OF THE SOY BEAN

While the interest of this report centers chiefly in the relation of soy-bean oil to paint manufacture, it seems advisable to indicate briefly the general chemical composition of the soy bean. A brief comparison between its composition and that of other oil-producing seeds has accordingly been arranged (Table 1).

TABLE 1.—*Chemical composition of various oil-producing seeds*

Seed	Source of data	Oil	Water	Ash	Crude protein	Fiber	N-free extract
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Soy bean	Henry and Morrison (7)	17.5	9.9	5.3	36.5	4.3	26.5
Do.	Delaware Bul. 99 (*14*)	17.2	11.7	4.8	33.5	4.5	28.3
Do.	U. S. Dept. Agr. Farmers' Bul. 58 (*9, 16*).	16.9	10.8	5.8	34.0	4.6	28.7
Flaxseed	Henry and Morrison (7)	33.7	9.2	4.3	22.6	7.1	23.2
Do.	North Dakota Bul. 118 (*15*)	37.5	5.0	.....	24.6	.....	.....
Do.	....do	39.3	6.7	.....	.....	.....	.....
Cottonseed	Henry and Morrison (7)	19.0	9.4	4.6	19.5	22.6	24.9
Do.	Delaware Bul. 99 (*14*)	19.9	10.3	3.5	18.4	23.2	24.7
Dent corn	Henry and Morrison (7)	5.0	10.5	1.5	10.1	2.0	70.9
Sweet corn	....do	7.9	9.3	1.8	11.5	2.3	67.2
Sunflower	....do	24.7	6.9	3.1	16.1	27.9	21.3

The soy bean averages 17 to 18 per cent oil, which is considerably lower than flaxseed and much higher than corn. Analyses reported by Fellers (*3*) for a large number of soy bean varieties are given in Table 2. These analyses show that there is a distinct varietal dif-

ference not only in percentage of oil but also in percentage of protein. Some well-known varieties like Auburn, Manchu, Guelph, Black Eyebrow, and Medium Yellow yield high in oil as well as in protein. This is an important consideration where soy beans are used for feed as well as for oil. Incidentally, Fellers believes that the oil is more easily pressed from some varieties than from others.

TABLE 2.—*Average percentage of oil and protein in different varieties of soy beans; from Fellers (3)*

Variety	Number of samples	Average per cent of oil	Average per cent of protein	Variety	Number of samples	Average per cent of oil	Average per cent of protein
Manchuria	3	23.2	36.9	Haberlandt	6	19.2	38.3
Auburn	3	21.6	40.0	Ito Sân	6	19.0	41.4
Manchu	6	21.6	40.5	Wilson	6	18.8	37.3
Ohio 9035	6	21.5	37.3	Manhattan	4	18.5	37.9
Guelph	8	21.2	38.1	Peking	6	17.9	37.3
Black Eyebrow	172	20.2	38.7	Hollybrook	6	17.2	40.8
Medium Yellow	6	19.6	39.3	Arlington	6	16.7	43.4
Early Brown	16	19.5	40.3	Ebony	8	14.6	43.6

It has been reported that an average bushel of soy beans contains about 11 pounds, or 1.42 gallons, of oil. At present oil mills are expressing only 70 to 75 per cent of the total amount of oil contained in the beans. By the proper use of a solvent, such as benzol, practically all of the oil might be removed.

The oil of the soy bean normally is practically neutral, and about 95 per cent is saponifiable. It is very similar to linseed oil. The oil itself consists of about 95 per cent of glycerol esters of the fatty acids. The oleic, linoleic, and linolenic acids are the unsaturated acids that are responsible for the drying quality of the oil. Their various proportions are given in terms of percentage as follows:

Oleic acid	26.5 (Smith). 33.4 (Baughman and Jamieson).
Linoleic acid	56.6 (Smith). 51.5 (Baughman and Jamieson).
Linolenic acid	2.4 (Smith). 2.3 (Baughman and Jamieson).

The oil of the soy bean is classed as a semidrying oil, being more efficient than cottonseed oil and less so than linseed oil. After treatment with certain driers, it is satisfactorily mixed with linseed oil if the proportion of soy-bean oil does not exceed 20 to 25 per cent.

Published data on the iodine values of soy beans, indicating the drying quality of the oil as compared with other oils, are recorded in Table 3.

The average iodine number of soy-bean oil can probably be given as 128 to 130, although ordinary commercial oil often tests somewhat lower. Linseed oil is markedly higher, and cottonseed and corn oil are much lower. There is considerable variability among the different varieties of soy beans in their iodine value as well as among different samples of the same variety. In general, it is true that there is no significant difference between imported and domestic beans.

TABLE 3.—*Iodine numbers of oil of different seeds*

Oil	Source of data	Number of samples analyzed	Iodine number
Soy bean:			
Domestic	Delaware Bul. 99 (*14*)	48	129.7
Do	Paint Manfrs. Assoc. Circ. 37 (*10*)	5	125.8
Imported	....do	2	128.8
Do	Paint Manfrs. Assoc. Circ. 67 (*4*)	7	130.7
Do	....do	9	134.5
Ito San	North Dakota Bul. 118 (*15*)	17	131.2
Haberlandt	....do	8	129.2
Wilson	....do	17	129.0
Manchuria	....do	31	126.6
Mammoth Yellow	Baughman and Jamieson (*1*)		128.0
Linseed	Delaware Bul. 99 (*14*)		180.0
Do	Paint Manfrs. Assoc. Circ. 67 (*4*)	3	189.3
Do	North Dakota Bul. 118 (*15*)	48	185.9
Cottonseed	Delaware Bul. 99 (*14*)		108.0
Corn	....do		119.2

PREVIOUS WORK ON THE HEREDITY OF CHEMICAL CHARACTERS

Except for experiments on plant pigments and considerable practical breeding for sugar in beets, there has been little direct research on the inheritance of chemical characters in plants.

The Illinois corn-breeding experiment (*12*) on selection for high and low protein and high and low oil content stands preeminent in this field. By means of simple mass selection, four strains of corn were isolated after 10 years of breeding, a high-oil and a low-oil, a high-protein and a low-protein strain.

By analyzing the pedigree records of the Illinois experiment, Surface (*13*) was able to show that the selection process had merely isolated already existing types of oil and protein percentage, the intermediate types having been discarded during the years of selection. This process is characteristic of an open-pollinated crop like corn.

Pearl and Bartlett (*11*) describe a preliminary undertaking on the heredity of chemical characters in maize. From their experiments they infer that such seed characters as moisture, nitrogen, protein, crude fat, ash, crude fiber, pentosans, sucrose, dextrose, and starch are inherited in maize essentially in accordance with Mendelian principles. They state that "probably each of the characters, protein, crude fat, and ash content, segregates as a definite and distinct unit character." However, their evidence is limited and their conclusions remain unchecked. All present evidence indicates a far more complex situation. Especially is this true of protein, as the work of East and Jones (*2*) proves.

Woodhouse and Taylor (*17*) report some interesting and critical results obtained in experiments on the relation between nitrogen content and oil content of soy beans. In these experiments they were apparently working with pure varieties, and their analyses are based on seed from single plants.

The varieties or types of soy beans used were named according to seed-coat characterietics, black, yellow, and chocolate. The vegetative characters of the yellow and chocolate types were similar, but

distinctly different from those of the black type. A summary of the findings that bear on the present problem follows:

Variety	Nitrogen, per cent	Oil, per cent	Correlation, nitrogen and oil
Black	6.72±0.02	13.52±0.08	−0.25±0.07
Yellow	5.61± .02	16.99± .07	− .34± .07
Chocolate	5.57± .02	17.13± .09	− .33± .07

It is evident that the black variety is distinctly different from either of the other two, both in nitrogen and in oil content. This difference is statistically significant, as the writers have determined by calculating the probable errors of the differences. For example, between the black and yellow varieties there is a difference in nitrogen of 1.11 per cent, the probable error of which is 0.03 per cent. For oil content the difference between these varieties is 3.47 per cent, with a probable error of 1.05 per cent.

It is interesting to note that a slight negative correlation exists between nitrogen and oil content in all three varieties. In other words, a high oil content apparently means a slightly lower nitrogen content. Woodhouse and Taylor stress the point that the correlation is not very large. They are inclined to believe that "the percentage content of nitrogen and oil is in all probability a characteristic that is inherited." They also note that selection for extreme percentages of nitrogen and oil did not affect the chemical content of the progeny in these respects in the following year.

Fellers (*3*) is inclined to believe that, in general, varieties with a high percentage of protein (or nitrogen) show a slightly lower percentage of oil. By calculating the coefficient of correlation for protein and oil from his data for 25 varieties, a fairly high negative correlation is found (-0.56 ± 0.09). But in view of the nature of the material, which was a heterogeneous mixture of varieties, the value of such a correlation is extremely uncertain.

Similarly, from certain data given by Grantham (*6*) the writers have calculated the correlation between percentage of protein and oil. These data are given in the form of chemical analyses for 51 varieties of beans, a single determination for each variety. To determine a correlation in such a mixture of types is often hazardous, but in view of Fellers (*3*) and Woodhouse and Taylor's (*17*) results in a similar situation, these data seemed to afford an interesting check. The correlation between these two characters was found to be -0.02 ± 0.09. This negative correlation is so small, however, especially in comparison with its probable error, that no evident relation can be said to exist between percentage of protein and percentage of oil.

Ladd (*8*), in chemical analyses for oil quality and percentage of oil in the soy bean, finds that the iodine number of a certain soy-bean varity is somewhat changed by the locality in which the plants are grown. Limited data indicate that "when beans giving a certain iodine value in one locality are then grown in a colder climate they show a decided increase in the iodine number. When soy beans from the North are grown in a warmer climate the iodine value drops." He also states that "an increase or decrease in iodine value

seemed to have no consistent effect on the oil content nor does the oil content seem to affect the iodine number." These conclusions have an important bearing on the results of the investigations reported herein.

It is worthy of note that both the results of Garner, Allard, and Foubert (*5*) and of Fellers (*3*) show that early and late plantings of the same variety produce some differences in the percentage of oil. The very late plantings showed a decrease in oil percentage. The first-named investigators are not inclined to regard the difference as due primarily to the date of planting, however, but rather to the character of the season itself. They found no correlation between size of seed and percentage of oil. The addition of phosphorus to pot cultures seemed to increase the oil content, but potassium had no effect. Under practical conditions, they were inclined to believe that climate is a more important factor than soil type in affecting the oil content of soy beans.

Fellers (*3*) remarks that in his experiments phosphates increased the protein content of soy-bean seeds at the expense of oil and that lime (oxide or carbonate) depressed the oil content. He also maintains that the inoculation of soy-bean plants with *Bacillus radicicola* caused a marked decrease in oil content.

EXPERIMENTAL RESULTS OF SELECTION FOR OIL QUALITY

As a preliminary step in the selection experiments, 11 varieties or strains of soy beans from various sources were analyzed in 1912 for their iodine number and percentage of oil. The results of these preliminary tests are recorded in Table 4.

TABLE 4.—*Chemical analyses of oil in soy beans of different varieties in 1912*

Variety or B. P. I. No.[a]	Number of plants analyzed	Average per cent oil	Average iodine number	Range in per cent oil	Range in iodine number
Wisconsin Brown	5	18.0	127.6	17.4 to 18.8	122.2 to 132.5
17257	5	16.8	133.1	12.6 to 19.2	127.4 to 136.3
17258	6	19.3	128.8	17.2 to 23.6	124.2 to 133.7
17268c	4	18.1	131.2	17.1 to 18.9	122.2 to 137.7
17277	5	19.0	123.6	17.2 to 21.0	109.9 to 132.4
18227	6	17.3	130.1	14.0 to 20.0	125.3 to 137.8
20405	5	17.3	129.6	13.9 to 19.1	121.0 to 139.6
20406	5	19.9	130.1	18.4 to 21.3	126.0 to 133.2
20854	7	20.1	122.4	17.7 to 22.1	117.0 to 128.0
Illinois Yellow	2	17.8	128.2	16.5 to 19.2	127.6 to 128.9
Wisconsin Brown	4	19.1	126.0	18.0 to 20.2	122.2 to 128.0

[a] Bureau of Plant Industry, U. S. Department of Agriculture.

As a result of these analyses, B. P. I. No. 17277, which showed the greatest range of variability in iodine number, was chosen as a starting point for the selection experiments. In view of the complex variability of this variety in the succeeding years, it would perhaps have been wiser to choose a variety that was purer for its morphological characters.

The seed of No. 17277 was obtained from the department of agronomy at the University of Wisconsin, having come originally

from the Bureau of Plant Industry of the United States Department of Agriculture. The variety name at that time was Manhattan. The seeds were yellow, with traces of brownish seed-coat pigment around the hilum. This variety was far from pure, for there was variation in flower color (purple and white), in pubescence color (tawny and gray), in growth habit (tall and dwarf), and in time of maturity.

Five plants of No. 17277 were analyzed in 1912. Of these, one plant, Wisconsin pedigree No. 1225c, which analyzed highest in iodine number (132.4) was chosen to continue the selection experiment.

The seed of 1225c was grown in 1913. Again variability of the strain appeared, especially in flower color and growth habit. Chemical analyses of 20 plants taken at random among the progeny were made, and the results recorded (Table 5).

TABLE 5.—*Iodine number and percentage of oil of progeny of original plant 1225c, 1913*

Plant No.	Iodine number	Per cent of oil	Plant No.	Iodine number	Per cent of oil
1325c–100	133.0	17.7	1325c–88	126.7	17.5
1325c–142	131.5	18.1	1325c–148	126.7	17.2
1325c–136	130.5	18.4	1325c–90	126.3	17.8
1325c–110	130.5	19.2	1325c–80	125.6	17.7
1325c–64	130.2	19.5	1325c–58	124.6	17.2
1325c–112	129.5	17.0	1325c–84	124.5	20.0
1325c–122	129.5	17.9	1325c–62	124.3	17.6
1325c–144	129.5	18.4	1325c–152	117.1	18.9
1325c–138	128.0	18.7	1325c–132	107.5	14.2
1325c–164	127.6	19.3			
1325c–60	127.1	18.7	Average	126.5	18.0

Although considerable variability in iodine number was manifest, only one plant surpassed the analysis of the mother plant. This plant, 1325c–100, with an iodine number of 133, was consequently chosen as the starting point of the high line. The plant lowest in iodine number, 1325c–132 (107.5), was chosen to serve as the beginning of the low line.

From this point selections for high and low iodine number were made in each of the following years: 1914, 1915, 1916, 1917, 1918, 1919, and 1920. In each year the plant of the high line having the highest iodine value was selected to continue the high line, and the plant of the low line having the lowest iodine value was used to continue the low line. Thus selections were made on iodine value and not on percentage of oil, although the latter was always determined.

The average (mean) yearly iodine number with its probable error, and the variability of each year's progeny as measured by the standard deviation, are listed in Table 6.

TABLE 6.—*Means and standard deviations of eight years' data for high and low oil selection in soy beans in terms of iodine number*

Pedigree No.	Year grown	Number of plants analyzed	Selection line	Mean (average) iodine number	Standard deviation
1325c-100	1913	1	High	133.0	----------
1325c-132	1913	1	Low	107.5	----------
5	1914	20	High	137.1±0.5	3.1±0.3
6	1914	20	Low	135.9± .3	2.3± .2
99	1915	14	High	125.4±1.2	6.5± .8
98	1915	8	Low	131.4± .6	2.3± .4
207	1916	50	High	130.2± .4	3.8± .3
212-3	1916	20	Low	128.4± .4	2.4± .3
400	1917	87	High	134.5± .2	2.4± .1
401	1917	44	Low	132.2± .2	2.3± .2
866	1918	61	High	136.6± .4	4.6± .3
867	1918	45	Low	124.9± .2	2.2± .2
1129	1919	34	High	131.9± .5	4.2± .3
1131-2	1919	41	Low	124.5± .3	3.3± .2
1325	1920	63	High	132.5± .3	3.5± .2
1331	1920	16	Low	125.2± .8	4.5± .5

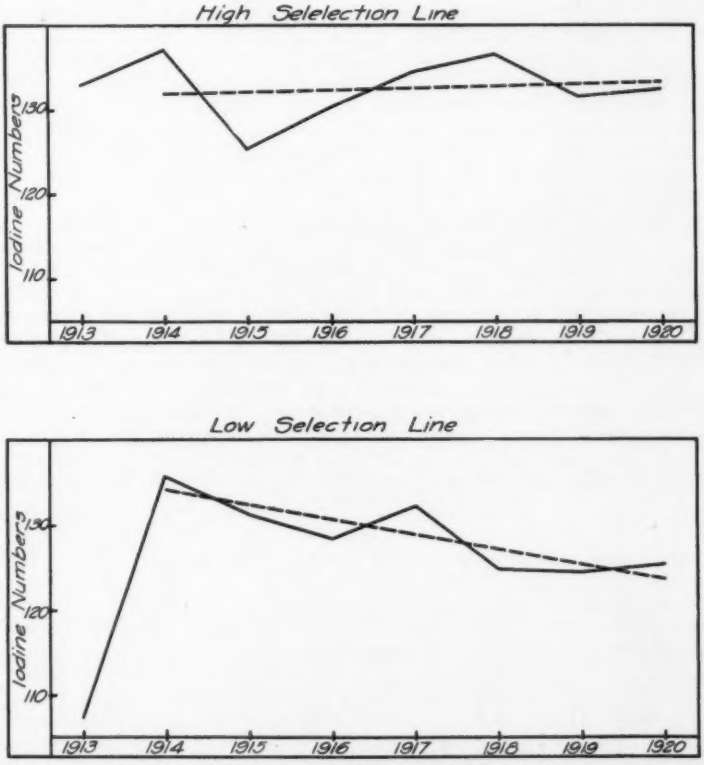

FIG. 1.—Yearly averages of iodine numbers in the high and the low selection lines of soy beans for a period of seven years (1913-1920). The broken line is a fitted straight line to indicate general trend

The general trend of the eight years' data on selection for iodine number is most easily visualized by plotting graphically the yearly averages, as has been done in Figure 1. In this graph the high and low lines have been plotted separately, and a straight line has been fitted to the data by the method of least squares. This line should indicate the general trend of the results, which is somewhat obscured by seasonal or yearly fluctuations such as are common with all determinations of chemical characters in plants. In fact, as will be shown later, these fluctuations are a serious obstacle to any selection work, since they often obliterate the true genetic nature of the plant.

The general trend of the data on iodine values for the high line during the seven years of selection seen in Figure 1 shows a slight upward tendency. It indicates that some slight progress has been made in increasing the iodine number of the variety. But the increase is so small that its real significance might be somewhat in question. When compared with the original average of 123.6 (Table 4), however, there is perhaps no doubt that the increase is significant. From a commercial standpoint such an increase is of doubtful value, since it has not surpassed the already existing quality of soy-bean oil to an appreciable extent.

A glance at the lower half of Figure 1 reveals a distinct downward tendency of the low line. If all the data are considered (including the abnormally low value of 107.5 of the single plant from which the line originated) a fitted line shows a slight upward trend. This obviously does not fit the facts of the case, for this upward tendency is entirely occasioned by the first ordinate in the graph. This point, which is that of only a single-plant analysis (and that most certainly an abnormal one, as shown by the distinct jump in the following year 1914), should not be permitted to influence the rest of the data to such an extent as it does. Accordingly, a straight line has been fitted to the data of the years 1914–1920, each ordinate of which is represented by an average of a fair sample of analyzed plants and not of a single plant. When this is done a pronounced downward trend is noted. This undoubtedly approximates the true condition.

In order to provide a more detailed analysis of the data, the differences between the averages (means) of the high and low lines for the successive years of selection have been arranged (Table 7).

TABLE 7.—*Difference between the average iodine numbers of the high and low selection lines*

Year	Selection line	Mean iodine number	Difference [a]	Year	Selection line	Mean iodine number	Difference [a]
1913	High	133.0	25.5	1917	High	134.5±0.2	2.3±0.3
	Low	107.5			Low	132.2± .2	
1914	High	137.1±0.5	1.2±0.6	1918	High	136.6± .4	11.7± .4
	Low	135.9± .3			Low	124.9± .2	
1915	High	125.4±1.2	−6.0±1.3	1919	High	131.9± .5	7.4± .6
	Low	131.4± .6			Low	124.5± .3	
1916	High	130.2± .4	1.8± .6	1920	High	132.5± .3	7.3± .9
	Low	128.4± .4			Low	125.2± .8	

[a] Any correlation existing between the two variables has been disregarded in calculating the probable error of the difference.

The last column in this table shows that during the early years of the experiment (1914–1916, inclusive) there was no constant or significant difference in iodine value between the high and low lines. Commencing in 1917, however, the difference attains a statistical significance in comparison with its probable error, and this is continued in the following years. In the last two years (1919 and 1920) the difference appears to have become constant. Certainly it is mathematically significant; and there is probably not much doubt that the high and low lines have been noticeably separated during the course of selection.

From the experimental data presented above one might deduce that the selection methods had actually changed the chemical constitution of the oil in the original variety of soy bean. But another phase of the experiment seems to reveal the underlying cause.

This new phase of the problem is concerned with a certain morphological or physiological character of the soy-bean plant and its apparent relation to the iodine value of the oil. It has been stated that the original variety from which the high and low selection lines were developed was, unfortunately, not pure for certain botanical characteristics, notably flower color, pubescence, maturity, and growth habit. Further, it has been noted that the selection process eventually rendered the two lines pure for certain botanical characters.

At the present time the high line is characterized by purple flowers, gray pubescence, lateness of maturity, and a tall, somewhat indeterminate habit of growth. The line breeds true for these characters. The low line, on the other hand, is pure for white flowers, gray pubescence, and a dwarf, stocky, determinate habit of growth. The low line is also fully two weeks earlier than the high. The distinction in growth habit is clearly seen in Figure 2, which shows a high and a low line row of plants growing side by side in 1922.

By 1916 the original mixture of botanical characters was straightened out as far as the low line was concerned. As to flower color, this low line proved to be white from the very beginning. The growth habit, which at that time was more difficult of determination because of variation, was not certainly pure until 1916, when it was noted as 100 per cent dwarf; and this was accompanied by an early maturity.

The high line, on the other hand, continued to throw the dwarf type in a definite proportion (approximately 25 per cent, being a simple Mendelian recessive) until and including the year 1919. In 1920, however, it was decided to render it pure for this growth habit. Accordingly, 10 of the highest-analyzing plants of 1919 were selected and grown in progeny rows the following year. Four of these proved to be 100 per cent tall. They repeated this process uniformly in the following seasons of 1921 and 1922.

As the matter now stands, these selections for plant characteristics, by removing the secondary modifiers of growth habit, have produced two unusually distinct and uniform lines of plants. The high line is remarkably uniform for growth habit (tall and somewhat indeterminate), purple flower color, and time of maturity, being distinctly late or medium late. During the season of 1922 evidences of mosaic rendered an otherwise vigorous strain somewhat unhealthy in appearance, but apparently did not influence the yield to any extent. This high line would be classed as a more than average yielder of seed.

The low line is characterized by a stocky, bushy habit of growth, white flowers, and very early maturity. It is a very poor yielder in

FIG. 2.—A, row of low-line selection of soy bean, showing early dwarf type; B, row of high line, showing the late tall type

most seasons. The low line beans show much greater development of a brownish pigment of the seed coat around the hilum than do those of the high line.

The point at issue, however, is the relation between these botanical characteristics and oil production. It has been noted that apparently the difference produced by high and low selection for iodine number (an average difference of about seven to eight in the last three years) was caused by a slight increase in the high line and a marked decrease in the low line. With this in mind a survey of the data should reveal something of interest.

A glance at Table 7 shows that in the years 1914 to 1917 there was no great difference in average iodine number between the high and low lines, except in 1915, when an actual difference in favor of the low line occurred (for which there is no accountable reason). There was an average difference of about two points in favor of the high line for the years 1916 and 1917. Later, in 1918–1920, this difference was markedly accentuated.

However, when the records, both botanical and chemical, are scrutinized carefully a possible explanation for this phenomenon appears. If the analyses within the high line are separated so as to group the late tall type of plant separately from the early dwarf type occurring in that line during those years, it is apparent that there are actually two groups chemically; the one with the higher analyses coming from the tall late plants and the other, which is considerably lower, from the early dwarf plants.

This fact is conclusively shown in Table 8, in which the iodine numbers of the late tall plants are contrasted with those of the early dwarf plants. The data are taken from the 1916, 1917, 1918, 1919, and 1920 crops, since before those years the distinction between the two plant types in the high line was not accurately enough determined to permit certain classification.

TABLE 8.—*Iodine numbers of late tall and early dwarf types of soy beans*

Year grown	Late tall type (all from high line)		Early dwarf type (from high and low line)		Difference
	Number of plants	Average iodine number	Number of plants	Average iodine number	
1916	44	130.5	26	128.9	1.6
1917	66	136.5	64	131.9	4.6
1918	51	137.9	55	126.2	11.7
1919	50	133.4	66	124.4	9.0
1920	63	132.5	15	125.2	7.3

It will be noted that, beginning in 1916, the late tall plants show a consistently higher iodine number than the early dwarf plants. This difference becomes marked in 1918, and from that point continues to be statistically significant. It so happens that the 1920 data show the least difference. The excess shown by the high line (132.5 ± 0.3)—which in that year consisted only of the late tall type—over the low line (125.2 ± 0.2), consisting only of the early dwarf type, is 7.3 ± 0.9. The difference is eight times its probable error, and is of real statistical significance.

From this we are forced to conclude that the separation of the high and low lines in the selection process is in all likelihood due pri-

marily to the isolation of a low-analyzing strain characterized by an early dwarf type of plant. Whether the correlation existing is between quality of oil and earliness (and lateness), or tallness and dwarfness is uncertain. The following indirect evidence seems, however, to throw some light on the problem.

Two sets of 10 plants each of pedigree 207 were chosen for analysis at different stages of maturity. About 10 pods were picked from each plant. The first picking was made on August 23, when the seeds and pods were green; the second on September 8, when the pods were turning yellow; and the third on September 18, after the plants were fully mature and the seeds thoroughly dry.

TABLE 9.—*Chemical analyses of soy beans at different stages of maturity*

Set No.	Date of picking	Average per cent of oil	Iodine number	Per cent of water, dry basis	Stage of ripeness
1	Aug. 23	19.06	124.9	71.6	Pods and seeds green.
1	Sept. 8	24.11	125.6	41.0	Pods turning yellow.
1	Sept. 18	18.41	128.8		Seeds ripe and dry.
2	Aug. 23	19.30	126.5	71.1	Pods and seeds green.
2	Sept. 8	24.17	124.6	40.3	Pods turning yellow.
2	Sept. 18	18.17	129.1		Seeds ripe and dry.

The analyses of the seed from these two sets of plants are shown in Table 9. The two sets check each other fairly well. It is significant that the iodine number increases quickly at the last stage of maturity, there being very little difference in the early stages.

One explanation which may be offered to account for the fact that the late, high line has a higher iodine value than the early low line is that the quick maturity of the low line may possibly retard the complete development of the unsaturated oils, whereas the slower maturity of the high line may provide better conditions for the oil formation.

A further explanation is offered in view of the experience of Ladd (*8*). He found that a colder climate markedly increased the iodine number of a variety, and that when northern varieties of soy beans were grown in the South, a noticeable decrease in the iodine value occurred. Accordingly, the cooler weather prevailing at the time the high line matured might probably have influenced the iodine value, since the lower temperatures occurred at a time when, as the experiments indicated, a rapid change in iodine value was taking place. The climate of Wisconsin becomes noticeably cooler during September, especially at night; so that a difference in maturity of two weeks at this time might perhaps be critical in the development of the unsaturated oils. For this reason it seems logical to assume that the correlation noted above exists between high iodine value and late maturity (or low iodine value and early maturity). If this assumption is true, the soy beans of highest quality must necessarily be the later-maturing sort, or they must be grown so that their critical stage of maturity coincides with cool weather. This is a matter that can readily be tested by experiment.[4]

[4] This point was subjected to a test in 1923, but because of adverse circumstances in the field, the value of the material for experimental purposes was destroyed. Attempts were made to verify the hypothesis later, but conditions prevented it. After much delay this report is presented without verification of this point.

VARIABILITY IN IODINE VALUES DURING THE PROCESS OF SELECTION

Ordinarily the continued selection for any one character tends to produce a more uniform progeny in respect to that character, and this is especially true of pedigree selection such as was practiced in this experiment. It is a noticeable fact, however, that the variability of the iodine numbers did not decrease materially as the selection process went on (Table 6). Perhaps the obvious reason for this inconsistency is the fact that a chemical character like quality of oil is extremely susceptible to external conditions. Climatic conditions for example, are known to exert a very marked effect upon it. Hence some of the variability shown in the early stages of the experiment might be attributed to such causes, and not all to the heterozygous genetic condition of the soy bean. Selection would then cause no appreciable change in the variability, especially after the plant type was fixed.

For this reason successful selection for high (or low) quality of oil is very difficult. The true genetic potentiality of the plant is so obscured by these temporary fluctuations caused by the immediate environment that the selection for high or low quality is rarely based on heritable variations that would make for progress.

TABLE 10.—*Means and standard deviations of eight years' data on percentage of oil in soy beans*

Pedigree No.	Year grown	Number of plants analyzed	Selection line	Mean (average) per cent of oil	Standard deviation
1325c–100	1913	1	High	17.7	
1325c–132	1913	1	Low	14.2	
5	1914	20	High	14.8±0.1	0.8±0.1
6	1914	20	Low	15.5± .1	.8± .1
99	1915	14	High	15.1± .2	1.0± .1
98	1915	8	Low	14.3± .2	.8± .1
207	1916	50	High	16.2± .1	1.4± .1
212–3	1916	20	Low	17.5± .1	.6± .1
400	1917	87	High	14.0± .1	1.5± .1
401	1917	44	Low	13.7± .1	.7± .1
866	1918	61	High	16.8± .1	1.1± .1
867	1918	45	Low	16.2± .1	1.4± .1
1129	1919	34	High	17.1± .1	1.1± .1
1131–2	1919	40	Low	15.4± .1	1.3± .1
1325	1920	64	High	16.7± .1	.7± .1
1331	1920	16	Low	14.6± .2	1.3± .1

A glance at Table 10 shows that the variability in oil percentage was also unchanged during the course of the experiment. This is not strange since there was no attempt to select on the basis of oil percentage.

It should be noted that the many morphological characters of the soy-bean plants were gradually rendered less variable during the eight years' time. Apparently external or climatic factors influence such characters much less readily than they do the chemical characters.

RELATION BETWEEN QUANTITY AND QUALITY OF OIL IN THE SELECTION EXPERIMENTS

As has been stated, while the selection for high and low quality of oil was in progress, the amount or percentage of oil per plant was likewise determined. The figures thus obtained offer an excellent criterion by which to detect any relation between quantity and quality of oil.

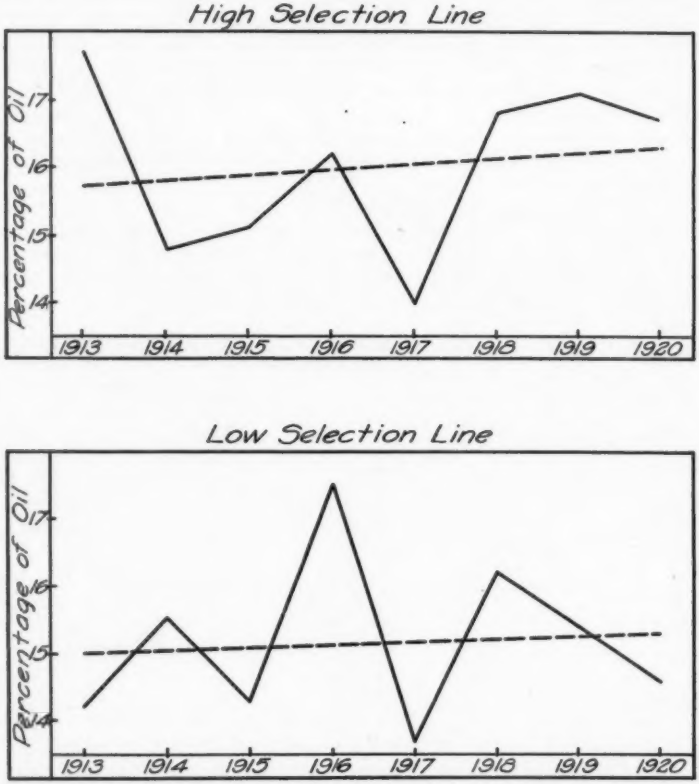

FIG. 3.—Yearly averages of oil percentage in the high and the low selection lines of soy beans for a period of seven years (1913–1920). The broken line is a fitted straight line to indicate general trend

The average oil percentages for the eight years of selection work appear in Table 10 and are graphically presented in Figure 3.

A glance at Figure 3 shows that there has not been a marked change in the average oil production per plant as the iodine numbers have increased or decreased. In both lines there is a very slight increase in oil percentage during the eight years. The average percentage of oil in the high-line material during the last three years is approximately 16.8, while in that of the low line for the same period

it is approximately 15.4. In other words, there is a difference between the two lines of over 1 per cent.

The exact differences in oil percentage are shown in Table 11. The original plants that served as the beginnings of the high and low selection lines happened to show a marked difference in amount of oil (17.7 − 14.2 = 3.5). This difference was not maintained during the early years of the experiment (1914–1916). But it is clearly evident that as the high and low lines began to draw apart in iodine number (beginning in 1916 or 1917), this difference in percentage of oil again became manifest. It is certainly significant in the last three years of the experiment.

TABLE 11.—*Difference between the average percentages of oil in the high and low selection lines*

Year	Selection line	Mean oil percentage	Difference	Year	Selection line	Mean oil percentage	Difference
1913	High	17.7	3.5	1917	High	14.0±0.1	0.3±0.1
	Low	14.2			Low	13.7± .1	
1914	High	14.8±0.1	− .7±0.1	1918	High	16.8± .1	.6± .1
	Low	15.5± .1			Low	16.2± .1	
1915	High	15.1± .2	.8± .3	1919	High	17.1± .1	1.7± .1
	Low	14.3± .2			Low	15.4± .1	
1916	High	16.2± .1	−1.3 .1	1920	High	16.7± .1	2.1± .1
	Low	17.5± .1			Low	14.6± .1	

The cause of the slight increase of oil percentage in both lines is difficult to explain. It may lie in the continued and unconscious selection for better plants, especially those with larger seeds and better yields. At least the experiment indicates that one may select for iodine value without decreasing the percentage of oil in the soy bean.

A verification of the above-noted relationship between percentage of oil and iodine number is afforded by a correlation study between these two characters. When the iodine values and percentages of oil are correlated the correlation coefficients do not indicate a noticeable relationship between these two characters.

TABLE 12.—*Correlations between percentage of oil and iodine number in soy beans*

From chemical analyses of—	Year grown	Correlation coefficient
Progeny of original plant	1913	−0.05±0.16
No. 17277	1913	+ .14± .09
No. 17277—duplicate	1914	− .20± .08
High-selection line	1916	+ .33± .09
Low-selection line	1916	+ .10± .15
High-selection line	1917	− .13± .08
Low-selection line	1917	− .06± .10
High-selection line	1918	+ .16± .09
High—duplicate seed of high 1918	1919	− .21± .13
Low selection line	1918	− .01± .10
High-selection line	1919	+ .17± .09
Low-selection line	1919	+ .32± .05
High-selection line	1920	+ .12± .08
Low-selection line	1920	− .42± .15
Composite sample of high line	1917–1920	− .13± .04
Composite sample of low line	1917–1920	− .27± .05

Correlation coefficients were calculated for these chemical characters over a period of years. The high-line data were considered separately from the low line. Within the high line only the late tall type of plant was considered, since a close relationship between the plant type and the iodine value has already been proved. Hence, beginning in 1916, when the differentiation between the late tall and the early dwarf type was apparent, the high-line data include only the former. A summary of the correlation coefficients appears in Table 12.

The striking fact in this table is the variability of the coefficients of correlation. In slightly more than half of the cases they are negative, in the other half, positive. The same seed gives a positive correlation one season and a negative correlation another. In some of the years the correlations are statistically significant but of low degree. It is true that the number of observations in any one year is very limited, but with so many years' data it seems perfectly obvious that no constant relationship between iodine number and percentage of oil can be said to exist. An unweighted average of the negative coefficients gives a value of $r = -0.16$, while a similar average of the positive coefficients is $r = +0.19$.

If one were permitted to average the entire list of correlations, the result would show none of any significant value. The fact that the data of both the high and the low line, when all the chemical analyses are grouped, show a negative correlation which might be statistically significant, means very little actually. Such a composite sample is affected more by the yearly relations of the chemical analyses to one another than by any inherent relation between the two correlated characters. This is but another indication that the climatic factors of the different seasons are extremely potent in their influence on the oil of the soy bean.

Considering the fact that the trend of the percentage of oil was not apparently affected directly by the increased and decreased values of the iodine numbers as the years went on (fig. 3), together with the lack of consistent correlation between oil percentage and iodine number, it seems reasonable to conclude that these two characters are not very intimately related to each other. One can select for an increased quality of oil without decreasing the quantity of oil produced by the soy-bean plant.

DISCUSSION

From the results of this investigation it seems clear that any improvement in the drying quality of soy-bean oil is not likely to come through continued selection in any one strain that has been purified by ordinary selection methods. There are two reasons for this. First, the ordinary variety or strain of soy bean is reasonably pure for its inherent oil characteristics. This is undoubtedly due to its method of self-pollination, which tends to isolate relatively pure (or homozygous) strains naturally, especially if the quality of the oil is correlated with a definite morphological or physiological character. Second, the quality of the oil (because of the nature and percentage of the unsaturated fatty acids) is readily influenced by external conditions (nonheritable), which tend to obscure any small, heritable differences that might exist and might be used in selection.

Accordingly, the quickest method of determining the best types of soy beans in respect to oil quality is to analyze soy beans of as many different varieties or strains as possible, and to select the highest-analyzing strain which is adapted to a specific region and which has proved to be a good yielder. Judging from all known reports, it is doubtful whether the iodine value of the soy bean can be raised to an average of 140. It may be that the highest average value now known, which is 134 to 135, represents nearly the physiological limits of the soy-bean plant. It is true that occasional analyses show values as high as 141 to 143, which might indicate further possibilities of the plant, but this is doubtful.

If selection methods are ineffective there remains the hope that hybridization may be effective in raising the iodine value. It is entirely reasonable to believe that by crossing two different vatieties, one might eventually combine in the progeny certain favorable factors for oil quality absent in one or the other parental varieties.

As regards quality and quantity of oil, the experiments reported in this paper give strong evidence that there is little or no interrelation. In other words, selection for high quality will not necessarily depress to any extent the quantity of oil produced by the plant. One may reasonably hope to isolate a high-quality variety that will yield not only a good amount of seed, but also a high percentage of oil. For example, the writers' high-line strain, which shows an average iodine number of 133.7 (three-year average), is classed as a good yielder with an average percentage of oil of 16.9 (three-year average).

As a result of these selection experiments certain general conclusions seem worthy of discussion. It was noted that the continued selection for high-quality oil produced a late-maturing strain, while the continued selection for low-quality oil resulted in an early strain. Data on the critical time for the formation of the unsaturated acids that are responsible for the quality of the oil indicated a point very late in the life history of the plant—just preceding maturity. A possible deduction was made that, in view of the fact that a cool climate seems to favor an increase in the iodine number, the late strain was higher in quality because it entered the critical period for oil formation when the weather was cooler. The low-quality strain, being fully two weeks earlier, matured when the weather was warmer.

If this situation proves to be a general phenomenon, it deserves considerable attention. The soy-bean oil industry at the present time is located in the Southern States, where cotton mills are used for expressing the oil. Obviously, this situation may not be the most efficient and economical one. Although the soy-bean oil industry is in a pioneer stage—at least as far as production is concerned—the huge amount of imported soy-bean oil indicates a vigorous growth of the market. According to a letter received in 1921 from the Bureau of Chemistry of the United States Department of Agriculture, "the larger part of the soy-bean oil used in this country is imported from the Orient. In comparison with the imported oil and beans, the amount of oil produced from domestic beans is practically negligible." Chemical analyses prove that domestic oil is of as high quality as imported oil.

The iodine value of oil, because of its intimate relation to the drying quality, is the most important standard by which to judge oils

intended for use in paints. In view of the variations in iodine number noted in these investigations, it will be necessary ultimately to buy oil on the basis of a standard test for quality, just as milk is now judged by its butterfat content and not merely by its bulk. For a drier in paint manufacture it is absolutely essential that high quality of oil be uniformly present.

SUMMARY

Continuous selection both for high and low quality of oil within one variety of soy bean for seven years produced a high and a low line differing to a significant extent in their average iodine number. An average of the last three years' data shows an iodine value of 133.7 for the high line and 124.9 for the low line. This is interpreted as the result of the isolation of two different genotypes within the original variety rather than of any change in a pure line.

The high line, selected entirely on the basis of chemical analyses, proved to be a late tall type with purple flowers, whereas the low line was early dwarf, and white-flowered. This was proof that the original commercial variety was neither a pure line for oil characteristics nor for season, growth habit, or even flower color.

Indirect evidence and some experimental data offer the interesting deduction that high quality of soy-bean oil is intimately correlated with a certain lateness of maturity. It is inferred that late maturity (in Wisconsin) provides the more favorable conditions for the complete development of the unsaturated acids that are responsible for high quality of oil.

Selection for high or low quality of oil proved to have no appreciable effect on the quantity or percentage of oil produced by the plant. This fact was verified by the lack of any consistent correlation between iodine number and percentage of oil. Correlation coefficients for these two characters taken over a period of years showed a high degree of variability but no consistent relationship. Such a situation makes it possible to select for high quality without decreasing the percentage of oil.

Large increases in the quality of soy-bean oil in any one variety by selection methods of breeding are not deemed possible.

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